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Serum Protein Fractions in the General Population:

Interrelationships of Paper Electrophoretic Patterns, Hemoglobin and Serum Uric Acid

Dennis G. Egnatz, B.A., cum laude,
Harvard College, 1962

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A thesis submitted in partial fulfillment of the requirement
for the degree of Doctor of Medicine
to
the faculty of the Yale University School of Medicine
Department of Epidemiology
March, 1967

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ACKNOWLEDGEMENTS

My thanks first to Professor Roy M. Acheson for the opportunity of working these past years with the problems of an interesting and extensive epidemiological study, The New Haven Arthritis Survey. The present thesis is based entirely on data from that Survey. I thank Dr. William M. O'Brien for his introducing me to the usefulness of computor techniques, without which this analysis would have been impossible. Also my gratitude for assistance in the final typing of this manuscript goes to Mrs. Emily Sprague, Mrs. Caroline Andrews, Mrs. Richie Haun and Mrs. Rosita Walsh, and to my wife, Jean, for the preparation of the drawings and large hand-drafted tables. Finally, my warm appreciation to all the many others with whom I worked who can only be recognized collectively as The Survey Staff.

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I. REVIEW OF THE LITERATURE

Introduction

Proteins in human serum represent a heterogeneous collection of essential substances which, for a given individual, remain remarkably constant in quantity, and for a given species also remain within rather narrow limits, although distinct differences are known to exist one species to the next (Moore, 1945). The medical literature contains numerous studies describing the alterations in these serum proteins found in many diseases (Brackenridge & Csillag, 1962; Fessel, 1962; Jencks, Smith & Durrum, 1956; Ogryzlo et al, 1959; Pollak et al, 1961; Putnam, 1960; Sunderman, 1964; Wall, 1958; and others), alterations which can be called "dysproteinemias" when the change is in the relative or absolute quantity of an otherwise normal protein component, or a "paraproteinemia" in which an abnormal protein, of distinct quality, is produced.

In the case of dysproteinemias, classification can be made either on the basis of disease systems and the protein changes they produce, or on the basis of the altered protein fractions or patterns and the associated diseases. In either approach, specific diagnoses are not usually made on the basis of serum protein changes alone, with the obvious exceptions such as analbuminemia or hypogammaglobulinemia. On the other hand, most paraproteinemias (multiple myeloma, for example), although rare in incidence, usually do have pathognomonic serum protein changes which lead to the specific diagnosis.

This study does not deal with the effect of disease on serum protein composition, but rather the serum protein composition of a population of New Haven adults living at home, representing all

I. ORIGIN OF THE SPECIES

Introduction

socioeconomic categories, but specifically not selected for their state of either health or disease. As far as is known, this is the largest general population with the possible exception of that of Samson (1964) in the Philippines, in which an electrophoretic analysis of serum proteins has been undertaken. Like all populations, however, some of its characteristics are probably unique.

Chemical Methods of Protein Fractionation

Prior to 1937, the study of human serum proteins was limited in clinical medicine to variation in either total protein, or later, to variation in the amount of serum albumin present in relation to that of serum globulins, commonly known as the "Albumin/Globulin or A/G Ratio". Total blood protein levels were at that time determined by Kjeldahl nitrogen assays which had to be corrected for non-protein nitrogen content.

In 1903, Reiss had made the first systematic investigation of the changes in refractive indices encountered in solutions of various blood-serum proteins. He used ammonium sulfate solutions varying from 32-50% saturation to precipitate serum proteins, and called his fractions "Euglobulin", "Pseudoglobulin I" and "Pseudoglobulin II". His salting out process, however, required crystallizations and dialyses which took some two to three months, and produced total protein values which were less than the sum of the various fractions.

Robertson, in 1912, reconsidered the fractionation process described by Reiss and was able to achieve a total protein value which equalled the sum of its fractions, which he referred to as "insoluble" and "soluble" globulins (equalling total globulins) and total albumins. Cullen and Van Slyke (1920) accepted Robertson's conclusion that ammonium sulfate was the most satisfactory salt for globulin precipitation and developed a technique which yielded uniformly consistent results in albumin and globulin fraction determinations by use of the Kjeldahl nitrogen-determination method in the final steps. This produced reliability in the determinations, but Howe (1921) offered two objections to this work of Cullen and Van Slyke: (a) the ammonium salt had to be

Prior to 1917, the study of boron ester pyrolysis was limited to clinical medicine to evaluate in minor local pyrolysis, or later, to materials in the manner of esters although pyrolysis is related to that of boron glycolites, commonly known as the "diboronic/dioxolin" ring opening". Small ethyl pyrolate levels were the time determined by electrophil nitrogen boracic valid had to be separated the monofluoric nitrogen content.

In 1921, Bates had made the first experimental investigations of the change in reductive lactone monomerized to solutions of various ethyl-boron pyrolates. The used ammonium solute solutions acrylic from 12-252 monomeric to pyrolytic boron esters, and called its reactions "Diglycolic" "Monoglycolic I" and "Dimonoglycolic II". His writing out pyrolate, however, required crystallizations and dilution which took more for three months, and produced total pyrolic values that were less than the sum of the native fractions.

Subsequent, in 1922, pioneered the investigation pyrolyzed borylene by Bates was able to monitor a total pyrolic value which showed the use of its fraction, which is related to be "pyrolytic" and "boric" glycolite (monolithic cotal glycolate) and total aluminum. Cited and Van Slyke (1930) accepted Bates's conclusion that monomeric and the most satisfactory salt for glycolic pyrolization and pyrolate was a tungsten white yielded medicinal examinations results in alumic and glycolic fraction determinations by use of the glycol ether-decomposition method in the first stage. This produced trimethyl in the diisopropylene, but Knox (1931) offered two objections to this work of Cullen and Van Slyke. (a) the monomolar salt had to be

removed prior to determining the globulin nitrogen because the nitrogen in the ammonium was a pollutant, and (b) that there were physical difficulties in removing that nitrogen. In turn, Howe proffered the substitution of sodium sulfate for the ammonium sulfate and produced a method for determining the serum albumin quantity which has continued in use up to the present day with only minor alterations. This albumin quantity, which when subtracted from the total protein value gives the globulin quantity, and these two fractions have come into common clinical usage regarding serum proteins as the A/G ratio.

The A/G ratio does, indeed, reflect relative changes in these two major classes of serum protein, or as Robertson (1912) wrote: "If the proportion of this substance (total globulin precipitated) is different in the serum of different individuals or species, we may be fairly confident, therefore, that the quantitative relations of the globulin and albumin, groups are different in these animals." However, even by 1921 Howe had concluded:

"Whether or not results obtained by precipitation of proteins from a mixture of proteins with salts represent separations of pure proteins is an open question. The considerable mass of literature on this subject is in favor of the opinion that the protein thrown down is a mixture of proteins; (a) present as compounds, (b) due to the absorption of other proteins by the precipitated protein, or (c) because the precipitation limits overlap."

limits overlap."

precipitated protein by the precipitated protein, or (c) because the precipitation is the result of (a) protein as compounds, (b) due to the adsorption of other proteins; (a) present as compounds, (b) due to the adsorption of other

"Whether or not results obtained by precipitation of protein from a mixture of proteins with salts represent separations of pure proteins is an open question. The considerable mass of literature on this subject is the cause of the opinion that the globulin fraction from a mixture of proteins is not necessarily that existing. In fact, Osborne postulated the subtraction of sodium sulfate for the edestin and proposed a method for determining the serum albumin quantity which has continued in use up to the present day with only minor alterations. This albumin quantity, which was ascertained from the total proteins using the globulin quantity, and these two fractions have come into common clinical usage regarding serum proteins as the A/G ratio.

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Electrophoresis

From the time that Sir William Hardy (1905), who was the first to observe the electrophoresis of proteins, studied serum with a migration apparatus, the great experimental difficulties of serum protein electrophoresis prohibited significant progress in that field. It was in 1937 that the Swedish investigator Tiselius, in connection with some research on proteins of immune sera, constructed a new electrophoresis apparatus specifically for the study of serum globulin.

Serum proteins, when in a solution of adjusted hydrogen ion concentration (pH) acquire a net surface charge which differs one protein to the next. When a mixture of such proteins is subjected to a continuing electrical field, the individual proteins will migrate at a rate characteristic of their net surface charge. This mild treatment of proteins is much less likely to introduce major changes in the protein molecules than is the rather violent chemical treatment of the salting out process. Tiselius applied this principle of electrophoresis to a solution of serum protein and found that serum could be separated into well-defined protein fractions which he called albumin and alpha, beta and gamma globulins (Tiselius, 1937b). This technique of studying solutions of proteins became known as "moving boundary" or "free" electrophoresis, and was a significant refinement in the separation of serum proteins. It did also prove that the globulin thrown down in the salting out process used previously was indeed heterogeneous. However, because of the elaborate and expensive equipment required, the difficult optical recording methods of ultraviolet photography used, the well-trained personnel needed, and the time consumed in each analysis, this moving boundary electrophoresis technique was available only at the research level.

For these reasons, there is but a paucity of literature prior to 1950 regarding serum protein electrophoresis. In that year, application of the principle of moving boundary electrophoresis to a solid medium system by Cremer and Tiselius, Durrum, and Turba and Enenkel greatly simplified the technicalities in terms of equipment, procedure and time. In place of a tube containing protein solution, they substituted strips of filter paper kept saturated with electrolyte solution and whose ends were kept at different electric potentials. The heterogeneous serum protein samples, placed at an intermediate position along the strip, were thus allowed to migrate along the filter paper and to separate into more or less discrete bands or zones. This type of electrophoresis has become known as "zone electrophoresis", in distinction to the "moving boundary" or "free" type described earlier by Tiselius.

Once the proteins had separated into zones, they could be heat-fixed and then stained with various protein-binding dyes. In general, the quantity of protein present and the intensity of the dye reaction bore a direct relationship to one another, so that relative amounts among the various protein fractions could be determined. This might be done either by cutting the strips and eluting the protein distributed along each section, or by direct photometric scanning of the paper strip and integration of the areas under the dye-intensity curve. For quantitative results it was still required that the total protein be determined by independent means and then absolute values be proportioned out on the basis of electrophoretic results for each fraction. The relative ease of operating such an electrophoresis process, the facility with which recording systems were directly coupled into the operation, the fact that several strips could be run simultaneously, and the added advantage that by using specific dyes on different strips from the same

sample one could place mucoprotein or lipoprotein, for instance, in relationship to the usual protein fractions determined, all made the electrophoresis process widely available to both research and clinical medicine.

Population Samples Studied by Paper Electrophoresis

In view of the serum protein changes which have been detected in various diseases, the majority of serum protein electrophoresis studies have concentrated their descriptive interest on the alterations which occur in the abnormal state. In large measure, the control samples used are pooled sera or sera from persons presumed or determined to be relatively healthy and to lie within a limited range of age. The five largest series of controls in serum protein analysis by means of filter paper electrophoresis among Caucasians are outlined below in Table 1.

Only in the two recent Scandinavian studies tabulated was an attempt made to study a wide range of adult ages among Caucasians. Some criteria of selection were exercised, and Kirkeby (1966) used careful screening to include only persons in good health, whereas Nilsson (1964) made random selections within a community of a wide range of ages based on birthdays on two calendar dates for the years 1884-1943, and then studied all those cooperating who had neither diabetes mellitus nor disabling disease.

It is evident that only the sample of 207 individuals chosen at random by date of birth (Nilsson, 1964) fully represents the age or sex proportions of a normal population. The New Haven study of 1029 individual adult sera offers an opportunity to investigate in a large population sample the basic biological information revealed through relatively simple paper electrophoresis of serum proteins in light of age and sex, and, because some Negroes were included, race also. Furthermore, it is possible in this sample to study correlations with other body chemistry parameters such as serum uric acid and hemoglobin.

In view of the serum protein changes which have been observed in various illnesses, the ability of these procedures eventually could have considerable significance in the clinical laboratory. Before such determinations can be made, however, the normal range of variation must be established. In large measure, the control samples used are pooled sera or sera from persons presumed or determined to be relatively healthy and to lie within a limited range of age. The largest series of controls in serum protein analysis by means of filter paper electrophoresis among Caucasians are outlined below in Table 1.

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Table 1

Normal or Control Series In Previous Serum Protein Studies In Whites Using Paper Electrophoresis

Reference	n	Source of Control Series
Acheson 1962	201	Males aged 65-85, random selection among pensioners
Jencks 1956	a) 4 serum pools	a) Pooled sera from presumably healthy, non-professional donors (mostly males)
	b) 185	b) Hospital patients with diagnosis presumed not to affect serum protein
Kirkeby 1966	170	92 males, 78 females hospital staff, industrial workers, and some over 60 excluded from blood bank roles because of age. No major disease, current menstruation or pregnancy. Lab and EKG studies for screening out abnormals.
Nilsson 1964	207	109 males, 98 females aged 20-79 selected at random from community. General condition good; previous diseases tabulated.
Ogryzlo 1959	100	Active; young adults aged 18-45 years in good health (hospital staff)

Table 2

Blood Donor Eyes Characteristics

Reference	n	Income at Control Decline
Anderson 1913	321	Rules acid Hill, routine relaxation observ Pennsylvania
Jones 1988	41 years	41 Dental care from previously Smalley, non-professional donors (mostly males)
		41 Dental patients with diagnosis presumed not to affect serum protein
Gibbons 1994	118	41 males, 31 females Surgical staff, industrial workers, and some over 60 enrolled from Alcoa test Dakota Research of age. No major disease, current smoking by screening was abnormal.
Gibbons 1994	197	182 males, 92 females aged 20-72 Veterans at random from community. General condition good; previous diseases Endocrinal.
Cyrus 1998	18	Kelvin young adults aged 21-41 years in good health (hospital staff)

Age, Sex and Serum Proteins

As might be expected from the nature of control samples previously noted, only a few authors have been able to comment upon age and sex with relation to serum protein, and fewer still have used the technique of electrophoresis in their analyses. Milam (1946), using semi-micro-Kjeldahl methods, determined total protein and albumin in over 1500 individuals and concluded that age and sex differences are obviously minor. Keltz and Comstock (1959) determined albumin and globulin in 197 sera by the biuret method and found that female globulins are higher than in males, but at a level not statistically significant, and that neither albumin nor globulin varies with age. They point out, however, that since the control sample was matched for age, sex and race with a group of sarcoidosis patients, the predominance in their sample of Negro females between the ages of 20 and 50 years is not otherwise found in a general population. Reviewing previously reported normal serum protein series done by the moving boundary technique of electrophoresis, and adding also a study of their own on professional and non-professional blood donors, Reiner et al (1950) stated that "age or sex differences do not affect appreciably the protein distribution in normal human serum".

Using paper electrophoresis analysis in 163 male and 22 female blood donors, Jencks, Durrum and Smith (1956) report that there is little sex difference in serum protein levels, and that "there is no great change in the electrophoretic distribution of serum proteins over the age of fifteen". However, their graph of total protein and protein fractions (expressed as a per cent of total protein) by age groups (Jencks, et al, 1956, Fig. 2) does suggest some variation which may be on the basis of

either age or total protein level. For example, total protein (in grams/100 ml.) and albumin (in per cent of total protein) are inversely related to one another at all points along the age curve in that study, with the relative albumin level reaching a peak in late middle-age and then falling off in subsequent years. If one were able to examine absolute quantities of albumin from their data, it might be found that in late middle-age the serum levels remain equal to or less than the levels in younger age, and that the fall in relative albumin at the oldest ages when the mean total protein is increasing might in fact reflect a significant decrease in the absolute quantity of albumin.

Among those authors who do find a difference in serum proteins when age or sex are considered, the most consistent conclusion reached is that serum albumin significantly decreases with increasing age. Bing et al (1946), using chemical methods for analysis of total protein, albumin and globulin in 87 males and females between ages 2-67 years, found that males 35 and older have slightly lower albumins, higher globulins but total proteins little different than males less than 35 years old. In females similarly grouped at 35 and older, the total protein is lower than in the younger females, with an increase in the variability of both the albumin and globulin fractions without any distinct change in the means for the two fractions. Thus they report a significant difference in serum protein by age (with 35 years the dividing age) and sex, although it seems with regard to sex they refer more to a difference in pattern of variability than in actual mean values. Wills and Bell (1951) report on 1,072 total protein estimations done in Fiji and Samoa by the copper-sulphate specific gravity technique that show there is no difference by sex, but that total protein increases

steadily in different age groups from infancy to 15-30 years, after which it falls off. Unfortunately, their five age groups of under 2, 2-5, 6-14, 15-30 and over 30 years do not continue through other adult ages, but the study does point out a steady, significant progression of serum protein levels during the years of growth and early adulthood.

Among the studies in the literature using electrophoretic techniques and finding age or sex associated with differences in serum protein levels in adults, Acheson and Jessop (1962) studied 201 retired men aged 65-85 and found gamma globulin levels higher than those reported on samples of predominantly middle-aged individuals. They also found that the level of gamma globulin in those men aged 75-79 is significantly higher (at the 2% level) than the level in those 10 years younger, and that the level in those 70-74 is intermediate. Brackenridge and Csillag (1962) studied 100 presumably healthy, white Australian males and females using cellulose acetate as the medium for electrophoresis of serum proteins and found virtually no variation in mean values with sex, but did find a small significant age trend in which albumin tended to fall and alpha-1 and beta globulins (the major lipoprotein fractions) tended to rise with increasing age.

In 1965 Samson et al reported from the Philippines on serum proteins from 1,005 non-hospitalized individuals ranging in age from the newborn period through adulthood. As in the present study, they used the Spinco paper electrophoresis system, although the present study does differ by using lissamine green rather than bromphenolblue as a protein dye, and bovine serum albumin rather than Versatol-A as a protein standard. Various age and sex differences in serum protein are described by Samson and his co-investigators. The following comments are restricted to

ages 20 years and older, because they are most pertinent in a comparative sense to the present study.

Samson et al report that serum albumin is greater in males than in females in all but the age groups 50 and older, and that each sex shows a steady decrease in albumin with age except for a transient rise in females aged 50-59 years. Total protein is greater in females aged 20-29, plateaus below male levels between 30-49, surpasses male total protein between 50-59 and finally falls to the lowest level for any age or sex group after 60. Male total proteins fall and rise between ages 20-49 before steadily tapering through the oldest group. Alpha-1 globulin means are similar in both sexes and show little trend with age. Alpha-2 globulin tends to be slightly higher in males except over 60 when the female means exceed those of the males. With respect to age, alpha-2 globulin rises in males to a plateau for the ages 30-49 before tapering while the females show no mean alteration between 20-49 before lowering slightly and then rising to their highest adult levels after reaching age 60. Beta globulin levels are higher in females only in ages 20-29 and show irregular changes with age, while male levels increase steadily between 20 and 59 and then remain constant after age 60. Gamma globulin is higher in females from ages 20-49, after which male levels exceed them for the remaining ages. In females the gamma globulin level rises from ages 20-49 and then tapers somewhat, while in males the level increases with each older group.

Another study using the Spinco method of paper electrophoresis and bromphenolblue staining was done in Norway by Kirkeby (1966) to compare blood lipids, lipoproteins and serum proteins between vegetarians and a group of controls ranging in age from 18 to over 60 years. His 170

controls (92 males, 78 females) were ambulatory, had had for the most part x-rays and annual health examinations, and were further screened for fitness with laboratory tests, blood pressure determinations and electrocardiograms to eliminate all those with major disease. Furthermore, no blood samples were taken from women who were menstruating at the time, were pregnant or who had given birth within one year. Among these control subjects, Kirkeby found no significant difference with respect to sex when means, regressions with age and variances were studied for total protein or any of its fractions, with one exception. In the beta globulin fraction the mean in men (0.94 g/ml) is significantly higher ($t = 2.91$, $p < 0.01$) than that in women (0.87 g/ml). Furthermore Kirkeby reports that the beta lipoproteins (specific staining) in his control series show an age variation similar to that for cholesterol, lipid phorphorus and total lipids with an increase up to the 40-49 year old group in men and up to an older group in women. The variation in beta lipoprotein with age is very highly significant among women, and is not significant in men when all age groups are included, although the correlation among men is highly significant when only the age groups 18-49 years are considered.

The Kristianstad Survey in Sweden (Nilsson, et al, 1964) was an extensive study of a random population sample of 207 normal adults (109 males, 98 females) using various clinical, anthropometric and laboratory tests with a special interest in the oral glucose tolerance test, but also including paper electrophoresis of serum proteins with an LKB apparatus and bromphenolblue stain. This appears to be the one sample population bearing the most similarity to that of the present New Haven study with respect to age, sex, race and state of health, the former

study excluding only 18 persons of 301 originally registered on the basis of either diabetes mellitus or disabling diseases contraindicating the performance of the examination planned.

When the serum proteins are studied with respect to age and sex in the Kristianstad Survey, the most pronounced finding is the negative correlation between albumin and age in each sex. Among the globulin fractions, alpha-2 and beta-2 show a strong positive correlation with age, and this latter correlation is even greater among males than in females. Total protein is higher in males in each of the three age groups (20-39, 40-59, 60-79), while albumin is slightly higher in females in the young and the old, but slightly lower than males in the intermediate age group. Females have a similar, but slightly increasing, level of alpha-1 globulins compared with males, and a higher alpha-2 level in the young females with no difference thereafter. Whether beta globulins are considered as a single or two separate fractions, male levels are higher than females in each age catagory except 20-39 when beta-1 globulins are similar in both sexes. Gamma globulins are the same in the two sexes in the youngest, higher in females in the intermediate, and higher in males in the oldest age group.

In summary, although several of the studies cited indicate that age and sex do not exert an influence on the variation in serum protein levels, the studies supporting the alternate conclusion gain weight in generally describing the same type of change with age and sex in the same fractions of protein. The changes with age are the more striking than those with sex, with albumin decreasing in older age and beta globulins increasing. To a lesser extent, alpha-2 and gamma globulins increase with age, while total protein and alpha-1 globulin do not vary

significantly. The difference described most often with sex is greater levels of beta globulin in males, and, less frequently, greater total protein, albumin and alpha-2 globulin found also in males.

Environment, Race and Serum Proteins

Studies of racial variation coming from regions quite different in nutrition and disease pattern from temperate climes have expectedly shown marked contrasts in serum protein levels reported. In 1951, Holmes et al, using chemical methods of fractionation giving results somewhat comparable to electrophoretic separations (Martin and Morris, 1949) report on 86 Africans from Kampala, Uganda the following differences from either American or Kampala Europeans: in Africans, total protein and alpha globulin are higher, albumin much lower, beta globulin lower, and total globulin and gamma globulin much higher. This can reasonably be explained by the poor nutritional intake which is particularly low in animal protein, the high incidence of liver disease (which is known to lower the albumin) and the high incidence of parasitic and other chronic infections (which elevate the globulin, especially the gamma globulin).

In Caracas, Venezuela (Vera and Roche, 1956) the electorphoretic patterns of serum proteins in 80 apparently healthy blood donors reveal total protein levels comparable to those in studies from the U. S. A., Chile, Belgium and Spain. Albumin levels are lower, however, and gamma globulins are markedly higher, although the gamma levels from Chile are somewhat more comparable. Differences among the several studies are not impressive for either alpha or beta globulins.

Wills and Bell (1951) studied total protein by the copper-sulphate specific gravity method in 823 persons (Fijian and Indian) living in Fiji and 249 persons (all Samoans) living in Samoa and describe distinctly higher total protein levels (up to 8.5 g/100ml) than those found in Europeans living in Europe or in Fiji, or in Indians living

Studies of initial metabolic rewarming from regional cold different-
ly modulated and diverse patterns from temperature client have experimentally
altered metabolic rate were previously reported. In 1811,
Bligh et al. using chemical analysis of thermoregulation clothes revealed
consistent comparable to electrophysiologic experiments (Dennis and Smith,
1849) reported on 89 citizens from Europe, Episode the Twilight-diffe-
rence from eighth dimensions or Bengali Interpersonal. In difficult, total
protein and alpha glycogen are higher, although much lower, these
glycolic lower, and total glycolic and plasma glycolic much higher.
This can reasonably be explained by the poor nutritional intake which
is particularly low in normal protein, the high tendency of liver
disease (which is known to lower the albumin) and the high intensity
of pancreatic and other glucose reabsorption (which always the glycolic,
especially the plasma glycolic).

In Caucasus, Venezuela (Rios and Banks, 1858) the electrophysiologic
patterns of various proteins in 60 experimentally healthy blood donors
versus tumor protein levels comparable to those in studies from the
U. K. a.. China, Belgium and Spain. Although levels are lower, hyperu-
cose, and plasma glycolytes are markedly higher, although the plasma
levels from China are accompanied more exaggerable. Differences among the
several studies are not interpreted for either higher or more glycolytes.

Mills and Bell (1811) studied total protein by the copper-salycyclic
glycolic precipitation method in 811 patients (Dillon and Jones) living in
Bill and 369 persons (all Japanese) living in Kansas and describe dis-
tinctly higher total protein levels (of as 0.3 g/100ml) than those

in India. Electrophoresis of serum protein was also done in a single healthy, young Fijian male and the gamma globulin was 2.3 g/100ml (29.0% of the 7.9 g/100ml total protein), a value which is more than twice that usually accepted as normal. The inference is that similarly high levels of gamma globulin might be found in many others from that same sample.

In a recent study (Samson et al, 1965) of approximately 1,000 apparently healthy Filipinos from different regions of the Philippines including the City of Manila and its suburbs, serum protein fractionation was done by electrophoretic methods almost identical with those used in this current study. In comparing the results in a similar age group with two other studies reviewed here including Venezuelans (Vera and Roche, 1956) and Caucasians and American Negroes (Pollak et al, 1961), the Filipinos have the lowest total serum proteins (7.22 g/100ml), alpha-1 globulins (0.16 g/100 ml), alpha-2 globulins (0.46 g/100ml), and beta globulins (0.63 g/100ml). Albumin for the Filipinos (4.55 g/100ml) exceeds that of American Negroes and of Venezuelans, but is second to that of Caucasians; and Filipinos have gamma globulins (1.41 g/100ml) higher than Caucasians and American Negroes but lower than Venezuelans. Table 2 below gives the values for the four groups.

Table 2

Serum Protein Pattern of Filipinos
Compared With Other Races

Races	Number	Total Protein	Albumin	Globulin Fractions			
				Alpha-1	Alpha-2	Beta	Gamma
Caucasian (30-50 yrs.) (Pollak '61)	62	7.49 ±0.51	4.60 ±0.94	0.29 ±0.06	0.61 ±0.126	0.86 ±0.127	1.13 ±0.23
Am. Negro (30-50 yrs.) (Pollak '61)	62	7.59 ±0.52	4.27 ±0.47	0.32 ±0.07	0.64 ±0.105	0.98 ±0.19	1.37 ±0.30
Venezuelans (Caracas) (Vera '56)	80	7.30 ±0.98	4.02 ±0.27	0.27 ±0.09	0.55 ±0.18	0.83 ±0.09	1.63 ±0.36
Filipinos (30-50 yrs.) (Samson '65)	165	7.22 ±0.55	4.55 ±0.61	0.16 ±0.07	0.46 ±0.16	0.63 ±0.18	1.41 ±0.44

Protein Units in g/100ml ± S.D.

Such analyses of racial variation are obviously wrought with major environmental differences. This is true even of those contrasting Caucasians living in the same area, as it is unlikely that such Caucasians have lived their entire lives in that same area, or if they have, that they have lived under the same conditions as the indigenous population studied. Schofield in 1951 made some attempt at evaluating what role environment played in the high gamma globulins and low albumins of native Africans. He studied by electrophoresis of serum proteins 30 West African men, all apparently healthy and between 17 and 34 years of age who were living in England at the time of the study. Most were students living reasonably well in both Africa and

Table 2

Serum Protein Pattern of Filipinos
Compared With Other Races

Race	Number Tested	Total Protein	Albumin	Globulin Fractions		
				Alpha-2	Beta	Gamma
Caucasian (30-50 yrs.) (Polish 'ol)	52	7.49 ±0.52	4.69 ±0.74	0.59 ±0.06	0.61 ±0.125	0.96 ±0.127
Am. Negro (20-40 yrs.) (Polish 'ol)	62	7.85	4.27	0.72	0.54	0.35
Chinese (Polish 'ol)	57	7.48 ±0.72	4.62 ±0.27	0.72 ±0.09	0.54 ±0.12	0.35 ±0.08
Filipinos (20-40 yrs.)105		7.32	4.55	0.76	0.46	0.83

Protein Units in g/100ml ± S.D.

Such analyses of racial variation are obviously open to with major environmental differences. This is true even of those contrasting Caucasians living in the same area, as it is unlikely that such Caucasians now lived their entire lives in that same area, or if they have, that they have lived under the same conditions as the indigenous population studied. Schofield in 1951 made some attempt at evaluating what role environment played in the high gamma globulin and low albumin of native Africans. He studied by electrophoresis of serum proteins 30 West African men, all apparently healthy and between 17 and 50 years of age who lived in Ibadan at the time of the [illegible]. Most were students living reasonably well in both Africa and

England, and eating mainly English food while in England.

The findings are that the greater the time which the students had remained in England without revisiting their African homes, the more similar the protein patterns became to those of Europeans. The changes were progressive with duration of stay up to eight years in the groups studied, and consisted of an increase in albumin to levels comparable to those in Europeans and a decrease in gamma globulins toward the levels in Europeans. Schofield concluded from this work that the difference in serum protein pattern found in Africa was not due to genetic or physiological factors, but to pathological changes which were at least partly reversible. He does, however, rely on three small groups of 10 for his statistics, and the gamma globulin levels in his Africans are still approximately 50% above his Europeans standards at the end of eight years. Although the data are suggestive of a significant environmental influence upon the albumin and gamma globulin difference between races, it is not altogether clear that his extrapolation is valid that gamma globulin differences would disappear with greater time.

Four studies done in the United States have compared serum proteins in Caucasians and Negroes. Although it is doubtful that total environment, either current or previous, would be the same for the two races, the similarities would be greater within a given city or area than would be when areas of entirely different climate are compared. The important concensus of these four studies is that, in spite of the elimination of many environmental differences, the serum protein racial differences show the same direction as those from other countries: serum albumin is lower in the non-white population, and serum globulins, particularly the gamma fraction, are higher in the non-white population.

With studies such as these it becomes increasingly difficult to ignore the likelihood that there indeed exists a racial difference in serum proteins which is genetic in basis.

In 1956, Rawnsley, Yonan and Reinhold conducted a blood-donor screening study to detect viral hepatitis carriers at the Hospital of the University of Pennsylvania. Thirty-six per cent of the Negroids and 63 per cent of the Caucasoid donors were born, and had always lived, in the Philadelphia area. Their ages ranged 18 to 59 years, and subjects who had history of jaundice, liver disease, syphilis or serious illness or a hemoglobin less than 12.5 gm.% were eliminated from their study. These authors used three methods of analyzing sera for gamma globulin levels: a) the Kunkell zinc turbidity method, b) ammonium sulfate turbidity, and c) zone electrophoresis (no details of this last method given). Their results are compiled in Table 3.

Table 3

Racial Differences in Gamma Globulin

Zinc Turbidity (Shank-Hoagland units) for Gamma Globulin

294 Negro males	7.32 ± 1.78	
826 Caucasian males	4.97 ± 1.44	Negro mean higher ($p < 0.001$) irrespective of age or sex.
38 Negro females	7.14 ± 1.72	
138 Caucasian females	5.38 ± 1.46	Negro mean 47% higher for gamma globulin

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Ammonium Sulfate Turbidity for Gamma Globulin

Caucasians	2.50 ± 0.16	($p < 0.01$)
Negroes	2.82 ± 0.23	

Zone Electrophoresis (45 Caucasians and 45 Negroes)

Negro mean serum albumin lower than Caucasian mean ($p < 0.05$)

Caucasian gamma globulin: 18.04 ± 4.41% of total proteins
Negro gamma globulin: 21.75 ± 5.09% of total proteins ($p < 0.01$)

[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

[illegible]	[illegible]	[illegible]

[illegible] [illegible] [illegible] [illegible] [illegible] [illegible]

[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

Comens (1957) reviewed 2,100 consecutive St. Louis hospital and clinic records (including those of 400 hypertensives and 1,700 others with miscellaneous conditions) and found 242 which had serum protein and protein fraction tests completed. He eliminated 70 of these 242 because of diseases known to alter serum globulins, and examined the results of the remaining 172. Of these 172, 83 were receiving antihypertensive drug therapy, so he divided his sample into 4 groups as follows: 44 White and 39 Negro hypertensives, and 61 White and 28 Negro normotensives. In addition to these groups he studied as normal controls 14 healthy, adult Negro male police officers who had undergone rigorous physical examination and training, and who on unrestricted diet which was comparable to that of the general American populace. However, no mention of the previous environmental background in these officers is mentioned.

Serum samples were all analyzed by the same laboratory, using the biurette method of Weichselbaum for serum protein determinations. In addition, on the group of 14 Negro officers, 48 filter paper electrophoretic analyses were done using the horizontal strip method (no details given) and the Spinco analyzer for the fractional interpretation.

Comen's results (Table 4) are that serum albumin levels are not significantly different when comparing any of the groups. Similarly, there is no difference between globulin levels when normotensive Whites are compared with hypertensive Whites, nor when normotensive Negroes are compared with hypertensive Negroes. However, when either any White group is compared with any Negro group, or the total whites are compared with the total Negroes, globulin levels in the Negroes are significantly higher ($p < 0.01$). The differences between the total Negro patients and the Negro officers are not significant for either albumin or globulin.

TABLE 1.—RACIAL VARIATIONS IN SERUM PROTEINS.

Group	No. Cases	Serum Albumin: Mean* Gm./100 ml.	Serum Albumin: Stand. Dev.	Serum Albumin: Stand. Error	Serum Globulin: Mean Gm./100 ml.	Serum Globulin: Stand. Dev.	Serum Globulin: Stand. Error	Elevated Values†: Albumin No.	Albumin %	Globulin No.	Globulin %
Negro Hypertensive	39	4.54 ± 1.05	.528	.0846	3.02 ± 1.48	.742	.1188	0	0	18	46.1
Negro Normotensive	28	4.50 ± 1.28	.641	.1209	2.88 ± .70	.350	.0660	1	3.5	7	25.0
White Hypertensive	44	4.60 ± .63	.317	.0477	2.43 ± 1.20	.603	.0908	0	0	4	9.0
White Normotensive	61	4.47 ± 1.05	.525	.0672	2.37 ± .83	.415	.0531	1	1.6	2	3.2
Total White	105	4.57 ± .978	.489	.0479	2.40 ± .99	.498	.0488	1	.9	6	5.7
Total Negro	67	4.53 ± 1.14	.573	.0700	2.97 ± 1.36	.682	.0833	1	1.4	25	37.3
Officers	14	4.64 ± .62	.481	.1280	2.83 ± .962	.314	.0830	0	0	5	35.7

* Standard deviation doubled

Standard deviation = $\frac{x^2}{n-1}$ where n = number of cases in that group
x = difference of mean and each case value

Standard error = $\frac{x^2}{n(n-1)}$

† Normal range = 2.0 — 3.0 gm./100 ml. serum for globulin
4.0 — 5.5 " " " " " " albumin

TABLE 2.—SIGNIFICANCE OF DIFFERENCES BETWEEN GROUPS

	Globulin t-ratio*	Globulin p	Albumin t-ratio	Albumin p
Normotensive Negro/Normotensive White	6.030	<.01	0.216	.8
Hypertensive Negro/Normotensive White	5.000	<.01	0.648	.5
Normotensive Negro/Hypertensive White	4.000	<.01	0.769	.4
Hypertensive Negro/Hypertensive White	3.940	<.01	0.617	.5
Hypertensive Negro/Normotensive Negro	1.020	.3	0.270	.8
Hypertensive White/Normotensive White	0.570	.6	1.577	.1
Total White/Total Negro Patients	5.800	<.01	0.471	.6
All Negro Patients/All Negro Officers	0.040	>1.0	0.075	>1.0

* Method of Fisher

Another important finding illustrated by the electrophoresis data on the 14 Negro officers is that a direct correlation exists between total globulins and the gamma globulin fraction which does not exist when the other globulin fractions were plotted against total globulins. This suggests that the high total globulin in Negroes is a result of a high gamma globulin alone, and that this hypergammaglobulinemia is a racial characteristic not dependent upon malnutrition, infection, liver disease, or hypertension.

Keltz and Comstock (1959) corroborated this hyperglobulinemia finding in studying serum proteins, by the biuret method in 75 White and 122 Negro controls (81% of the entire control population) from a sarcoidosis survey in Georgia and Alabama. Their population was not normally distributed in terms of age, sex and race in that they were matched in these characteristics with sarcoid patients, as mentioned previously. They found in the Negroes slightly lower albumin ($p < 0.06$), but significantly higher globulin ($p < 0.001$) than in the Whites. (Their results are in Table 5.) Furthermore, Negroes who at some time have had a positive serological test for syphilis (not necessarily active disease) are no different in globulin level from Negroes with no positive test history. With respect to tuberculin skin testing (5 units PPD-S) in the control population, although Negroes showed more skin induration and more total globulins, there was no relationship between the size of the tuberculin reaction and the level of serum globulin. The demonstration is that neither syphilis nor tuberculosis among Negroes could account for the high serum globulin levels consistently found, and that a racial differ- must be considered.

must be considered.

Table 5

*Serum Proteins in 75 Whites and 122 Negroes

	Whites	Negroes
Globulins:	2.3 ± 0.4	2.7 ± 0.5
Albumin:	5.0 ± 0.5	4.7 ± 0.5
Total Protein:	7.3	7.4

* (g/100ml ± 1 S.D.)

The final paper concerning racial differences in serum protein levels is the only report using filter paper electrophoresis to study both healthy Caucasians and healthy American Negroes living in the same city. This is a report from Chicago (Pollak et al, 1961) in which the electrophoresis methods differ from those used in the present study only in the use of bromphenolblue dye and a smaller current. In a systematic study of serum protein electrophoresis changes in patients with systemic lupus erythematosus, sera were obtained from 124 healthy persons for use as controls. The control sample was 62 Caucasians and 62 American Negroes, half of each group being men, the other half women, drawn mostly among healthy laboratory technicians, physicians and members of the hospital staff, and a few healthy blood donors. The majority were between the ages of 30 and 50 years, with a range of 20 to 75 years.

In using the electrophoretic technique they were able to extend the work of the previous studies to include the four globulin fractions now in common use, rather than limiting their observations to total globulins or less specific turbidity tests for gamma globulin. Their results (Table 6) clearly show a significantly lower serum albumin in the Negro group, as well as a higher gamma globulin fraction than for Caucasians, but they also show significantly higher alpha-1 and beta globulin

fractions in the Negroes, with no significant difference between the alpha-2 fractions.

Table 6

Serum Protein Fractions:*
62 Healthy Caucasians & 62 Healthy Negroes

	Total Protein	Albumin	Alpha-1	Alpha-2	Beta	Gamma
Caucasians	7.49 ±0.51	4.60 ±0.54	0.29 ±0.066	0.61 ±0.126	0.86 ±0.127	1.13 ±0.23
Negroes	7.59 ±0.52	4.27 ±0.47	0.32 ±0.072	0.64 ±0.105	0.98 ±0.19	1.37 ±0.30
"t"	1.08	3.63	2.66	1.21	4.11	4.87
p	0.2	0.001	0.02	0.2	0.001	0.001

*Mean results expressed in gm./100 ml. ± 1 S. D.

With the weight of evidence presented here indicating that there are racial differences in serum proteins, even when comparing racial groups in somewhat similar environments, it becomes obvious that the Negro sample within the New Haven Survey should be treated separately from the White portion of the sample. It had been originally intended to select only those areas devoid of Negroes for the reason that racial differences might affect the data. However, in the lowest socioeconomic areas it was impossible to delineate an area where unskilled white laboring families lived which had no Negroes. This presents a fortunate opportunity to study serum proteins in Negroes and Whites living in the same residential area, and for whom additional demographic and clinical information is known. Of the 85 Negroes (31 males, 54 females) participating in the laboratory examinations, 56 (18 males, 38 females) had complete electrophoretic analysis of their serum proteins. To

compare this group with the Negroes studied by Pollak, one can immediately say that: a) group size is similar, but more females are present in this study, b) age ranges are similar, c) both groups are in healthy state, although the New Haven sample will carry that range of health status compatible with everyday activity, although perhaps far from ideal, while Pollak's study simply states that all were in good health. This will, in any event, be only the second study available using filter paper electrophoretic methods to analyze serum proteins in healthy Negroes and Whites living under similar conditions, and will offer the added potential of more detailed knowledge about them.

Uric Acid, Hemoglobin and Serum Proteins

No population studies are reported in the literature examining both serum protein electrophoresis and serum uric acid. However, the report on the Tecumseh (Michigan) Community Health Study (Mikkelson, et al, 1965) reviews the age and sex relationships with serum uric acid found by other workers and describes results found in 6,000 individuals in that community. In considering uric acid in adults, previous authors noted significantly higher levels in males at all ages, and that uric acid levels either remain relatively constant with age or show only a slight rise with increasing age. In females, the results are more variable, with reports of a) no age effect between 30-79, b) a gradual increase in uric acid through all ages, or c) little variation until age 45 when levels begin to rise to approximate those in men.

In the Tecumseh Study the same significant sex difference is again documented, except at ages 44, 45 and 55-58 years. In adulthood the mean uric acid levels in males are at peak values between ages 20-24 after which they decline slightly to a plateau except for a slight later rise at 55-59 years. In females the age variation curve is distinctly different with an early slight decline and plateau until about age 40, then a slow rise from 40-54 years with peaks at 50, 54 and 60-64 years.

With respect to hemoglobin and serum protein electrophoretic patterns, there are two reports. The first comes from a study of Africans in Uganda (Holmes, et al, 1951) in which it is noted that in pooled data from 150 subjects the red blood cell count and chemically determined serum albumin correlate in a positive direction. At the same time total

protein falls slightly and serum globulin also falls as the number of RBC rises. In a further examination of 110 individual samples by chemical means of protein fractionation (complete data in only 86), it is found that the changes in total, protein, albumin and globulin remain as described above, that the alpha and gamma globulin fractions remain relatively constant with changes in the RBC count, and that the diminution of the total globulin with the increase in RBC is mainly accounted for by a fall in the beta globulin fraction.

In the Kristianstad Survey (Nilsson, et al, 1964) determined serum proteins by paper electrophoresis and also obtained values for hemoglobin, RBC count, and erythrocyte sedimentation rate in 207 individuals. Unfortunately, the data is not presented in such a way that the total protein and albumin means can be compared directly with hemoglobin, but certain correlation coeffieients can be noted. The correlation between beta-1 globulin and hemoglobin is significant but in a positive direction at the 10% level, and similarly for beta-1 and RBC at the 5% level. Other globulin fractions do not show similar correlations and those between albumin and hemoglobin are not given. The ESR does significantly correlate in a negative sense with each the albumin, hemoglobin and RBC, but the conclusion that albumin and hemoglobin therefore positively correlate cannot be made from the available information.

Mann (1966), in a Yale M.P.H. essay, examined the hemoglobin levels in 1201 individuals from this New Haven Arthritis Survey (1258 are used in the present study) and their relationships to each of the demographic categories which were used to describe the study population. There is no consideration of serum proteins, but in the analysis of variance, she finds several significant differences. The finding of higher

hemoglobins in males is clear. In males the analysis of variance in hemoglobin is significant for only social class, employment status and marital status. She suggests that these three factors may be interrelated in their effect on hemoglobin. The relationship between social class and hemoglobin in males does not follow a clear-cut trend; however, the analysis of variance shows that the means do vary significantly at the 5% level. Hemoglobin shows a very slight decrease with age in men, and White males have higher levels than Negro males, but the analysis of variance shows no significant difference.

In females, she finds age, race and marital status significantly associated with hemoglobin. Age has a significant effect on hemoglobin in both races at the 1% level when considering women under 45 (lower hemoglobin) as contrasted with those 45 and older. Race (hemoglobin higher in White females) has a significant effect at the 1% level in all age groups, in all except single and widowed women. The effects of age and race appear to be independent of one another, but are more pronounced in Negroes. Parity (in number of live births to a woman) appears to have no significant effect, but single women had significantly higher hemoglobins than nulliparous women who were either married, separated or divorced.

II. GENERAL OUTLINE OF THE SURVEY

The New Haven Arthritis Survey

The New Haven Arthritis Survey was begun in the Fall of 1960 with the purpose of collecting information on arthritis with regard to its prevalence in a population of New Haven adults representing the various socioeconomic groups in the city. Among other goals was a comparison of questionnaire, blood analysis, radiologic and photographic information as methods of screening for joint disease. This report is concerned solely with fractional analysis of the serum proteins as it relates to demographic data and blood analyses. The demographic data analyzed here are age, sex, social class and area of residence (which are related), and race and the laboratory data used are hemoglobin, serum uric acid, total serum protein and albumin by chemical determinations, and the serum globulin fractions alpha-1, alpha-2, beta and gamma by paper electrophoretic means.

The sample population was selected by social class, which was predicted from the census tract data on the basis of dwelling as described in the 1960 census. Six discrete areas of the city were thus chosen so as to have approximately 500 adults aged 21 and over in each of the social classes I to V (Class I being the highest). Then all persons aged 21 and over actually resident in these six areas were identified individually and became the study population.

The first phase of contact with the survey population was made in the home when a questionnaire was used, which could usually be completed within two minutes. Demographic information was gathered on age, sex, race, religion and, for social classification, the education and occupation of the head of the household. Social class was evaluated by

the Hollingshead and Redlich (1958) two-point classification scheme of education (weighted x 4) and occupation (weighted x 7) of the household head. There were also questions on symptoms and previous history of joint disease. In addition during this initial contact, the following were accomplished: the number and names of all persons in the household 21 or over were asked to confirm the original listing obtained from census tract information; a Clinistix was left with instructions for testing glycosuria; and an explanation and invitation to the survey mobile bus were offered.

The survey bus was the center of operation for the second phase of data gathering. The bus, described fully elsewhere (Acheson, et al, 1965), was borrowed from the city chest x-ray program and equipped and staffed, when needed, for the survey. Interviews not already done in the home were taken, Clinistix results were reported, time and type of last food intake were asked, blood samples were obtained by venipuncture, and x-rays of the hands and feet were taken. Blood samples were sent to the laboratory and analyzed for hemoglobin, blood group and type; ASLO (Anti-Streptolysin-O) titer, C-reactive protein level and rheumatoid factor were assayed, the last named by the latex fixation method; total serum protein, serum albumin, serum uric acid and blood glucose were assayed with an AutoAnalyzer; and serum globulin fractionation was carried out by paper electrophoresis.

Of the 2,345 adults actually enumerated in the survey population, 94% answered the first phase questionnaire supplying the demographic information used here, while the blood analysis data comes from the 61% (1,425) of the total population sample who had attended the survey

bus as of July 15, 1966. Although analysis for difference between groups attending and not attending the survey bus is not complete, we do know that with minor exceptions, the five social classes in those attending were each homogeneous with respect to: 1) uric acid

2) hemoglobin

3) morning stiffness

regardless of the area in which they lived. (Acheson, personal communication).

III. LABORATORY METHODS

Prior to the drawing of blood samples for laboratory determinations, persons were asked how long it had been since their last easting, and whether they had had breakfast, lunch, dinner or a snack. As about 1,200 of the 1,440 samples were obtained at the survey mobile bus, the exceptions being drawn at a special survey clinic, persons had been walking or standing immediately prior to sampling, and at variable outside ambient temperatures. With the subject sitting, a sample of venous blood was withdrawn with minimal delay, usually from an antecubital vein, into two separate vials, one with an EDTA anticoagulant for hemoglobin and blood group determinations, the other with no additive for determinations on serum.

Hemoglobin levels were determined by the standard cyanmethemoglobin method. Serum uric acid levels were determined upon dialyzed serum with the use of an AutoAnalyzer following Folin's phosphotungstate and cyanide method as adapted from Hawk, et al (1954). Specimens were exchanged at weekly intervals with the Public Health Service diabetes field study laboratory at Brighton, Massachusetts for comparison of uric acid, glucose and rheumatoid factor results, and the results here for uric acid were consistently 0.2mg/100ml higher than those obtained by their uricase method (Acheson and O'Brien, 1966).

Total serum protein and also serum albumin were determined quantitatively with the Technicon AutoAnalyzer, since the lissamine green stain used in the paper electrophoresis procedure is not reliable for quantitative measurement of the albumin fraction (see below). The biuret method was used for determination of total protein (Weichselbaum, 1946), standardized against crystalline Bovine Albumin, Fraction V,

III. LABORATORY METHODS

from Armour Pharmaceutical Co., Kankakee, Illinois. The bovine albumin standard curve for total protein compared quite closely with other curves based on crystallized human albumin (by Dade) or on Versitol. However, all results reported here are based on the bovine albumin standard.

Serum albumin was not separated from the total protein, but was quantitated on the basis of its dye-binding capacity with 2-(4'-hydroxyazobenzene) benzoic acid (HABA dye), also with the above bovine albumin used as the standard (Rutstein, et al, 1954; and Wrenn and Feichtmeir, 1956). Late in the chronology of the survey, other standards such as human albumin (Dade) and Versitol were compared, and the results reported on the basis of the bovine albumin were 0.5-1.5 g/100ml higher than with these other standards. With a total protein comparable among standards and an albumin higher with the particular standard used, the globulins are expectedly low, since the quantity of total globulins is here defined as the difference between the total proteins and albumin as determined with the AutoAnalyzer. Nevertheless, the albumin method was consistent throughout the survey, so that, even though absolute levels of albumin and globulins reported will contain this systematic error, other associations, correlations, and trends will still be valid.

The paper electrophoresis of serum proteins was done in a Spinco/Beckman model R system using a plastic Durrum-type vertical descending cell and Schleicher & Schuell 2043-A paper strips. Strips were allowed 15 minutes for equilibration with the B-2 Veronal Buffer, pH 8.6, ionic strength 0.075, before the 0.006ml serum sample was added. A constant current of 7.5 milliamperes was applied for 16 hours. Strips were then oven-dried at 65°C. Following drying, strips were pre-rinsed in purified

standard.

curves based on crystallized human albumin (Behring Institute, The Serum

Serum albumin was not separated from the total protein, but was

as the standard (Kunstein, et al, 1956; and Mrema and Peterhans, 1956).

(Dade) and Vertical were compared, and the results reported on the

basis of the bovine albumin were 6.3-6.9 g/100ml higher than with their

was slightly lower with the particular standard used, the globulins are

accordingly low, since the quantity of total globulins is here defined

as the difference between the total proteins and albumin as determined

with the refractometer. Nevertheless, the albumin method was consistent

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ations, corrections, and trends will still be valid.

The paper electrophoresis of serum proteins was done in a Spinco/

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cell and Schleicher & Schuell 2043-A paper strips. Strips were allowed

15 minutes for equilibration with the 0.1 Veronal Buffer, pH 8.6, then

strips 0.07N, before the 6.25Ma serum sample was added. A constant

current of 1.5 milliamperes was applied for 16 hours. Strips were then

oven-dried at 60°C. Following drying, strips were pre-cleaned in petrolatum

methanol for three minutes to remove buffer salts, and were then subjected to 0.3% lissamine green stain in 15% acetic acid for 10 minutes. After staining, excess dye was removed with a triple 6-minute rinse of 2% acetic acid solution until the strip ends were white, and the paper strips were blotted and placed in a 65° oven heat until dry. With lissamine green there was no need for a final exposure to ammonia vapors for color to develop before scanning, as with bromphenolblue dye.

The choice to use lissamine green as the protein stain was based upon work by Gorringe in 1957, when that author found this dye to satisfy most of the following criteria for suitability in paper electrophoresis work:

1) give consistent results from one batch to another,

2) stain protein but not paper,

3) stain all proteins equally,

4) retain a linear dye/protein relationship up to the maximum concentration of protein likely to be encountered,

5) be readily eluted in a non-volatile solvent,

6) be stable under conditions of use,

7) give the same results by elution and by direct densitometry,

8) be insoluble in clarifying agents used in direct densitometry,

9) be quick, cheap, and simple to use.

The notable exception to its suitability regards criteria 3 and 7 when using it for albumin, in which case it underestimates by about 17% (Gorringe, 1957) when scanning as compared with eluting. This discrepancy does not exist within the range of globulin concentrations normally encountered in human serum (ibid), and it is for this reason that paper electrophoresis was used only for proportioning of globulin fractions, while total protein and albumin levels were assayed using the methods described earlier.

Paper electrophoresis strips were scanned with transmitted light at 640 millimicra wavelength in a Densicord Densiomter (Model 542) which traced out the protein electrophoresis pattern according to the intensity of light interference at any given area. This unit was directly coupled with an Integraph Integrator which marked out a numerical value for all areas under the electrophoresis curve. Then, dividing the curve by globulin fractions alpha-1 through gamma, each fraction was assigned a value in g/100ml on the basis of its relative area under the curve multiplied by the g/100ml of total globulins as determined from the total protein-albumin difference separately determined by the AutoAnalyzer. Total protein and serum albumin were not determined by electrophoretic means in this study. Both the dyed strips and the plotted electrophoretic patterns are permanently stored.

IV. DATA PROCESSING

Recording

Data both from the questionnaire and from the laboratory analyses were consolidated for this study onto a single pre-coded form for each individual. This information was then punched and verified on standard 80 column IBM cards for analysis. Each individual card is identified by two separate numbers. The first, the survey number, is a four-digit number made up of the residential area (one to six) and three digits (001 to approximately 500) denoting consecutive order of survey contact within that area (e.g. 4-333). The second, the bus number, is a six-digit number denoting the date of visit (day of year plus last digit of year) and the number of the visit that day to the bus. (Example: 023-5-03 for 23rd day of 1965, the third person visiting the bus on that day.) Both of these numbers are on each IBM card used in the analysis, and no individual had more than one card or data from more than one visit used in the final analysis.

Recording

Data both from the questionnaire and from the laboratory analyses were accumulated for this study onto a single pre-coded data tape for each individual. This information was then punched and verified on standard 80-column IBM cards for analysis. Each individual card is identified by the appropriate numbers. The first, the survey number, is a four-digit number made up of the residential land (see 3a (2)) and three digits (000 to approximately 300) denoting sequentially order of survey received within that area (e.g., 0-121). The second, the bus number, is a three-digit number denoting the date of visit (day of year, plus last digit of year) and the number of the visit that day to the bus. (Example: 023-5-03 for 23rd day of 1965, the third person visiting the bus on that day.) Both of these numbers are on each IBM card used in the analysis, and on individual data sheets for each of the visits when more than one visit used in the final analysis.

Computation

All computer analysis was done directly from the card-recorded data. As preparation of the data cards had proceeded longer than one year's time with different workers involved, a data check was first undertaken. Using a SNOBOL program and the IBM 1620, the two separate identification numbers were searched for correspondence and duplications with the deck of cards. Among 1,445 bus numbers, 20 were thus eliminated for one of the following reasons:

1) person visiting bus was not living within the survey area,

2) person was from survey area, but was not at least 21 years of age, and therefore not eligible for the survey,

3) person was in survey, but had visited bus previously with completion of full set of tests.

Data on the remaining 1,425 cards were analyzed with the IBM 7094/7040 system by application of Yale Computer Center programs 31-S and 71-S. For computation of means, standard deviations, and correlation coefficients (program 31-S) among the 12 variables used, data cards were variously sorted (IBM card sorter) by sex and age groups for each race separately. For the multiple regression analyses (program 71-S), cards were first sorted for completeness in each of eight variables to be considered. Various multiile regressions were then carried out on both the data for White males (419) and White females (536) attending the survey bus.

All computer analysis was done directly from the card-recorded data. As preparation of the data cards had proceeded longer than one year's time multi-digit verification, a data check was not considered undertaken. Using a SCATOL program and the IBM 1620, the two separate files of data were compared for consistency and duplication with the check of data. Cards [illegible] for one of the following reasons:

1) person visiting bus was not living within the survey area,

2) person was from survey area, but was not at least 21 years of age, and therefore not eligible for the survey,

3) person was in survey, but not visited [illegible] of full age of data.

system by application of Yale Computer Center programs 31-S and 71-S.

(program 31-S) among the 12 variables used, data cards were variously sorted [illegible] by one and up [illegible]

sorted for completeness in each of eight variables to be considered.

Without multiple registration were then sorted [illegible]

the Optic sector (41%) and Optic Inserter (24%) alternating the survey

two.

Analysis

Results in mean values for the various race, sex and age groups are presented in this analysis together with a one standard deviation limit, except in the case of figures showing mean values graphed by age. In this latter instance, the means are plotted together with their 95% confidence limits (± 2 S.D.).

The Pearsonian correlation coefficients (r) are presented and described, and those levels of significance noted are derived from standard tables of statistics.

Data from the multiple regression analyses are presented in tabular form and briefly described.

V. FINDINGS

Demographic Data

Race and sex. Five categories of demographic information gathered through questionnaire response are considered, and they are:

1) residential area
2) social class
3) age
4) sex
5) race

Of the 2,345 adults actually enumerated in the survey population, 1,425 (60.8%) attended the survey bus and are considered in this study. Some of these had only radiography done, and blood sampling was not completed. Whites comprise 1,340 of this overall bus number (605 males, 735 females), and the remaining 85 are Negroes (31 males, 54 females). As an original goal of this survey was to hold constant the varisble of race by choosing an all-Caucasian population sample, the Negroes unavoidably included in the sample are studied as a separate group. There are also a few Puerto Ricans and four Orientals who are included in the White sample. The demographic catagories are, for the most part, complete for this sample. The laboratory analyses used in this report are incomplete in part for a minimum of 85 Whites (30 males, 55 females) and 5 Negroes (3 males, 2 females). Among the 1,340 Whites, data from the laboratory is complete for all variables considered in 955 individuals (419 males, 536 females) for 71.3% of that bus group.

Residential area. As has already been stated, for both area and social class, a rating of one reflects the high end of the socioeconomic scale.

Thus "mean area" and "mean social class" could be calculated by analyzing the mean values for these scores. It was necessary to select six areas for the five social classes in order to include social class one within the combined areas one and two in numbers which were comparable to those of other social classes available within a single area. Table 7 shows the mean residential area score for the various groups.

Table 7

Mean Score for Area by Race and Sex

Group	Mean Area ± 1 S.D.
1,340 Whites (both sexes)	3.66 ± 1.54
605 White males	3.64 ± 1.55
735 White females	3.69 ± 1.53
85 Negroes (both sexes)	5.86 ± 0.56
31 Negro males	5.97 ± 0.18
54 Negro females	5.80 ± 0.68
39 Negroes (both sexes) aged 21 to 34 years	5.90 ± 0.38
46 Negroes (both sexes) aged 35 to 76 years	5.83 ± 0.68

This table illustrates that Whites studied have a distinctly lower mean area number with much greater variability than do Negroes. (Of 85 Negroes studied, 78 live in area 6.) Furthermore, males and females within each race do not differ from each other with respect to residential area.

Among Whites, the positive correlation coefficients (r) between area and social class are highly significant (range r=0.477 to 0.766; $p < 0.01$) for all age groups (sexes either separate or combined) except for 16 females (mean age 78.7 years) for whom r (0.549) is significant

Table 1

Mean Score for Area by Race and Sex

Group	Mean Area ± 1 S.D.
1,340 Whites (both sexes)	3.65 ± 1.54
All White Males	3.84 ± 1.15
All White Females	3.46 ± 1.29
23 Negroes (both sexes)	4.84 ± 2.34
11 Negro Males	4.27 ± 2.14
12 Negro Females	4.92 ± 2.52
39 Negroes (both sexes) aged 21 to 34 years	5.00 ± 2.32
46 Negroes (both sexes) aged 35 to 76 years	5.80 ± 0.88

at the 5% level and for the age group 21-24 years (sexes separate or combined) for whom there is only a very low degree of correlation ($r=0.205$ to 0.014). As Pearsonian correlation coefficients (r) are not designed for non-continuous variables, the scores of area and social class are better studied by rank order correlation (rho). The value for rho in this sample is 0.7.

Correlations between area and age for Whites show an inverse correlation which is significant for females ($4=-0.070$, p about 0.05) and highly significant ($p < 0.01$) for males ($r=-0.169$) and for the combined sexes ($r=-0.115$).

Among Negroes, area and social class correlations are not significant in males or in the 35-76 year old group, but r is significant for females ($r=0.342$, $p < 0.05$) and highly significant for ages 21-34 ($4=0.527$, $p < 0.01$) and for all Negroes combined ($r=0.323$, $p < 0.01$), in a direct relationship.

The correlation between area and age is not significant for any Negro group studied.

Social class. As described above, the social class scale of one to five ranks the highest socioeconomic class as one. Mean values for the various groups, including white males and females by age, are given in Table 8.

Table 8

Mean Social Class Score by Race, Sex and Age

Group	n	Mean Social Class ± 1 S.D.
1,340 Whites (both sexes)	1,328	3.15 ± 1.41
605 White males	598	3.08 ± 1.39
735 White females	730	3.21 ± 1.42
605 White males by age group		
Range (Years) / Mean (Years)		
21-24 / 22.7	28	4.21 ± 1.34
25-34 / 29.7	97	3.51 ± 1.33
35-44 / 39.9	123	2.85 ± 1.35
45-54 / 49.7	165	2.71 ± 1.29
55-64 / 59.0	98	2.94 ± 1.36
65-74 / 68.7	67	3.42 ± 1.39
75- / 79.1	20	3.55 ± 1.43
735 White females by age group		
Range (Years) / Mean (Years)		
21-24 / 22.5	44	3.64 ± 1.28
25-34 / 29.7	117	3.43 ± 1.40
35-44 / 39.8	157	2.92 ± 1.31
45-54 / 49.2	192	2.90 ± 1.43
55-64 / 59.4	125	3.38 ± 1.39
65-74 / 68.7	79	3.63 ± 1.47
75- / 78.7	16	3.50 ± 1.51
85 Negroes (both sexes)	84	4.67 ± 0.80
31 Negro males	31	4.68 ± 0.54
54 Negro females	53	4.66 ± 0.92
Negroes aged 21-34	39	4.67 ± 0.87
Negroes aged 35-76	45	4.67 ± 0.74

Table 4.2

Mean Social Class by Sex, Marital Age

Group	n	Mean Social Class ± 1 S.D.
1,340 Whites (both sexes)	1,338	3.15 ± 1.41
605 White males	598	3.46 ± 1.38
735 White females	730	3.21 ± 1.42

615 White males by age group

Age (Years)	Mean (Years)	n	Mean Social Class ± 1 S.D.
21-24	22.7	28	4.21 ± 1.34
25-34	29.7	97	3.51 ± 1.33
35-44	39.9	123	2.65 ± 1.15
45-54	49.7	144	3.18 ± 1.28
55-64	59.5	109	3.84 ± 1.31
65-74	68.7	67	3.42 ± 1.35
75-	79.1	20	3.55 ± 1.45

735 White females by age group

Age (Years)	Mean (Years)	n	Mean Social Class ± 1 S.D.
21-24	22.5	44	3.64 ± 1.28
25-34	29.7	117	3.45 ± 1.40
35-44	39.8	137	2.92 ± 1.31
45-54	49.2	142	2.90 ± 1.43
55-64	59.4	125	3.35 ± 1.36
65-74	69.7	79	3.63 ± 1.47
75-	78.7	16	3.50 ± 1.51

85 Negroes (both sexes) 84 4.67 ± 0.86
74 Negro males 31 4.68 ± 0.56
54 Negro females 53 4.66 ± 0.92
Negroes aged 21-34 39 4.67 ± 0.87
Negroes aged 35-75 45 4.67 ± 0.74

As with the data on residential area, social class among the Whites is distinctly different from (lower rank value, higher socioeconomic status) that of the Negro as well as more variable. There is no sex difference for either race, and correlations with area are described above in that section. Correlation coefficients among Whites are only sporadically significant, but as seen in the above table, there is a trend in mean values for either males or females to higher socioeconomic status among the middle-aged groups. Among Negroes, there is no difference between any of the mean values, although the correlation coefficient between social class and age is highly significant for a single group, those 45 aged 35-76 years ($r=0.404$, $p<0.01$), probably a result of sampling.

Age. The following table contains the mean age values for the various groups.

Table 9

Mean Age by Race and Sex

Group	n	Mean Age in Years $\pm$ 1 S.D.
1,340 Whites (both sexes)	1,339	47.4 $\pm$ 14.3
605 White males	604	47.9 $\pm$ 14.4
735 White females	735	47.0 $\pm$ 14.3
85 Negroes (both sexes)	85	37.9 $\pm$ 12.5
31 Negro males	31	40.6 $\pm$ 12.0
54 Negro females	54	36.4 $\pm$ 12.7
39 Negroes (both sexes) aged 21-34	39	27.3 $\pm$ 3.9
46 Negroes (both sexes) aged 35-76	46	47.0 $\pm$ 9.9

Mean age values for Whites show little sex difference (males 0.9 years greater) and no difference in variability. There is, however, a 9.5 year greater mean value for Whites than for Negroes. Furthermore, Negro males average 4.2 years older than Negro females and 0.7 years less (1 S.D.) variability. It should also be noted that in the older Negro age group, while the range of age is 35-76 years, the mean age value of 47.0 years indicates a clustering towards the younger range.

Correlation coefficients between age and both area and social class are described under those headings.

Since the size (1,340) of the White sample allowed subdivision by age groups for analysis, the range, mean and standard deviations of age among males, females and combined sexes of these groups were easily derived by computer and are presented below for reference.

Table 10

Mean Age in Whites by Sex and Age Groups

Age Range (yrs.)	MALES n	mean ± S.D.	FEMALES n	mean ± S.D.	COMBINED SEXES n	mean ± 1 S.D.
21-24	28	22.7 ± 1.12	44	22.5 ± 1.13	72	22.6 ± 1.12
25-34	97	29.7 ± 3.10	117	29.7 ± 2.88	214	29.7 ± 2.97
35-44	123	39.9 ± 2.92	158	39.8 ± 3.06	281	39.8 ± 3.00
45-54	166	49.7 ± 2.77	195	49.2 ± 2.98	361	49.4 ± 2.90
55-64	102	59.0 ± 2.67	126	59.4 ± 2.90	228	59.2 ± 2.80
65-74	68	68.7 ± 2.71	79	68.7 ± 2.57	147	68.7 ± 2.63
75 & over	20	79.1 ± 3.99	16	78.7 ± 3.81	36	78.9 ± 3.86

Table 10

Mean Age in Whites by Sex and Age Groups

Age range (yrs.)	n	mean ± S.D.	n	mean ± S.D.	n	mean ± 1 S.D.
25-34	97	29.7 ± 3.10	117	29.7 ± 2.88	214	29.7 ± 2.97
35-44	125	39.9 ± 2.92	158	39.8 ± 3.05	283	39.8 ± 3.00
45-54	104	49.7 ± 2.77	195	49.2 ± 2.98	301	49.4 ± 2.90
55-64	102	59.0 ± 2.67	126	59.4 ± 2.90	228	59.2 ± 2.80
65-74	66	68.7 ± 2.71	79	68.7 ± 2.57	145	68.7 ± 2.63
75+	20	79.1 ± 3.99	16	78.7 ± 3.81	36	78.9 ± 3.88

Since the size (1,344) of the White sample allowed subdivision by age groups for analysis, the range, mean and standard deviations of age among males, females and combined sexes of these groups were easily derived by computer and are presented below for reference.

Correlation coefficients between age and both area and social class are presented under their headings.

Laboratory Data
(Mean Values and S.D.)

AutoAnalyzer results

Total serum protein and serum albumin. Mean values, together with S.D., of total protein and albumin are given below in Table 11 for both race and sex, as well as the two broad age groups in Negroes. Figure 1 gives the same information with 95% confidence limits (± 2 S.D.) for individual age groups among White males and females.

Table 11

Total Protein and Albumin by Race and Sex

Group	n	*Total Protein	*Albumin
1,340 Whites (both sexes)	975	7.49 ± 0.68	5.10 ± 0.56
605 White males	438	7.57 ± 0.67	5.25 ± 0.54
735 White females	537	7.42 ± 0.68	4.98 ± 0.55
85 Negroes (both sexes)	57	7.55 ± 0.70	4.90 ± 0.48
31 Negro males	19	7.43 ± 0.56	4.96 ± 0.41
54 Negro females	38	7.62 ± 0.76	4.86 ± 0.51
39 Negroes aged 21-34 yrs.	23	7.54 ± 0.88	4.86 ± 0.52
46 Negroes aged 35-76 yrs.	34	7.56 ± 0.56	4.92 ± 0.46

*Units in g/100ml ± 1 S.D.

Laboratory Data

(Mean values and S.D.)

Summary of Findings

Total serum protein and albumin (Bio-Science) Laboratories (Flow Electrophoresis) of total protein and albumin are given below in Table II for both race and sex, as well as the two broad age groups in Negroes. Figure 1 gives the same information with confidence limits ($\bar{X} \pm 1.96\sigma$) for individual age groups among White males and females.

Table II

Total Protein and Albumin by Race and Sex

Group	N	Total Protein	Albumin
White males (mean ages)	975	7.49 ± 0.68	5.10 ± 0.59
605 White males	478	7.57 ± 0.67	5.25 ± 0.54
725 White females	597	7.42 ± 0.69	4.96 ± 0.59
21 Negroes (Both sexes)	11	7.18 ± 0.87	4.80 ± 0.48
21 Negro males	19	7.43 ± 0.56	4.96 ± 0.41
34 Negro females	36	7.62 ± 0.76	4.76 ± 0.31
39 Negroes aged 21-34 yrs.	23	7.54 ± 0.85	4.86 ± 0.32
46 Negroes aged 35-76 yrs.	34	7.56 ± 0.53	4.92 ± 0.45

Figure 1

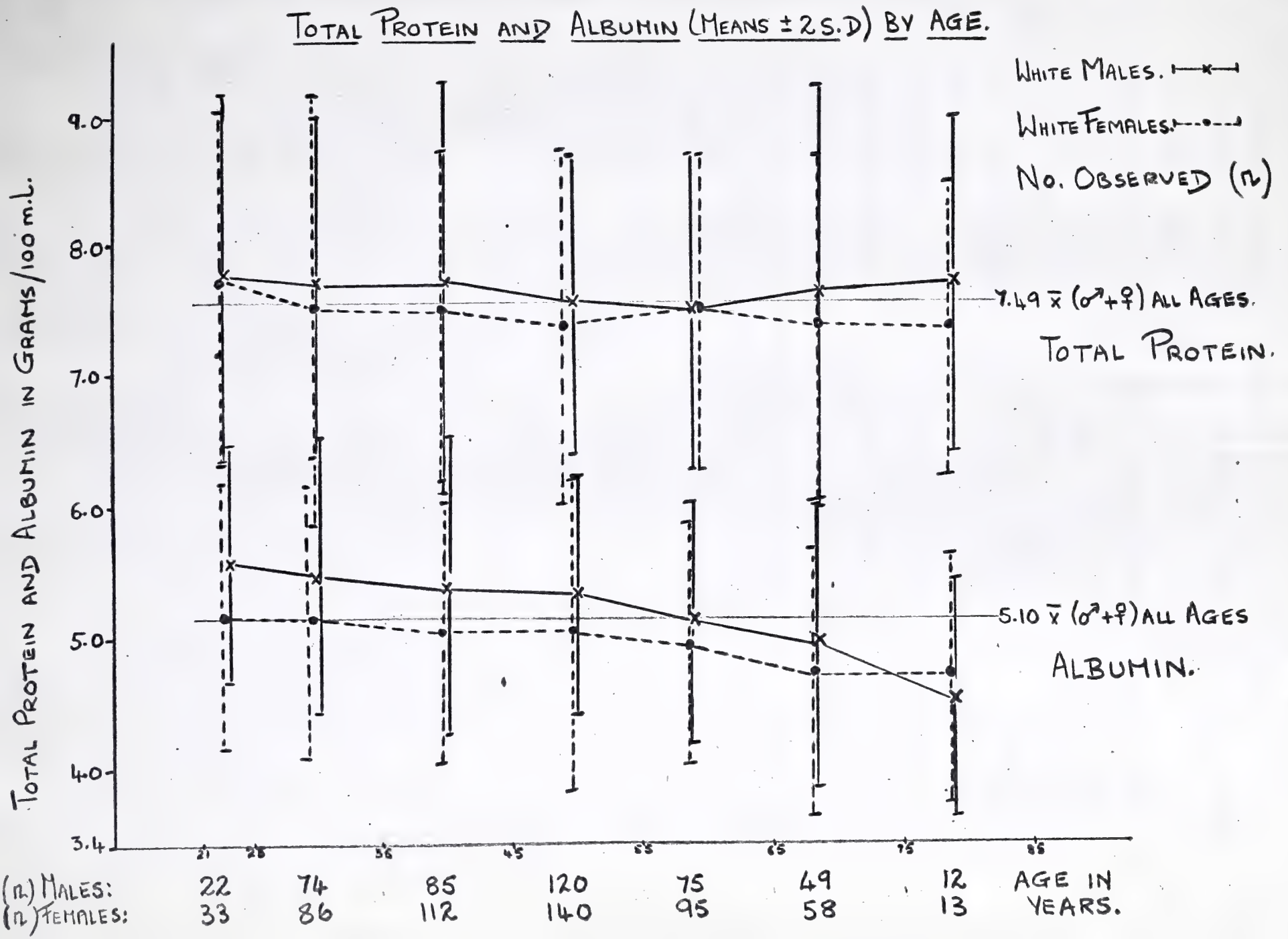

TOTAL PROTEIN AND ALBUMIN (MEANS ± 2 S.D) BY AGE.
WHITE MALES.
WHITE FEMALES.
NO. OBSERVED (n)
7.49 x̄ (♂+♀) ALL AGES.
TOTAL PROTEIN.
5.10 x̄ (♂+♀) ALL AGES
ALBUMIN.
TOTAL PROTEIN AND ALBUMIN IN GRAMS/100 m.L.
9.0
8.0
7.0
6.0
5.0
4.0
3.4
21
25
35
45
55
65
75
85
(n) MALES: 22 74 85 120 75 49 12
(n) FEMALES: 33 86 112 140 95 58 13
AGE IN YEARS.

Table 11 and Figure 1 illustrate that total serum protein are homogeneous when studied by age, sex and race. Serum albumin shows somewhat higher mean values in Whites when compared with Negroes (5.10 vs. 4.90 g/100ml) and in men of either race when compared with women of the same race (Whites: 5.25 vs. 4.98; Negroes; 4.96 vs. 4.86 g/100ml). The most notable finding is that serum albumin, when plotted against age in Whites, shows a marked and progressive trend towards lower albumin levels in the older age groups. This decrease in serum albumin is greater for White males (5.55 to 4.49) than in White females (5.14 to 4.67 g/100ml). The male albumin levels are 0.3-0.4 g/100ml greater than females between the ages of 21 and 54, after which age the downward slope of the male curve changes more rapidly than the female slope and the two means become more similar. Finally, in the small groups aged 75 and older (12 males, 13 females), the male value falls below the female mean value.

Table 11 and Figure 1 illustrates that total serum protein are homogeneous when stratified by age, sex and race. Males always showed slightly higher mean value in Watts when compared with Negroes (7.15 vs. 6.95 g/100ml) and Blacks when compared with women of the same race (Whites: 5.25 vs. 6.95; Negroes; 6.96 vs. 6.86 g/100ml). The most notable finding is that serum albumin, when plotted graphical sign in Nigeria, shows a marked and progressive trend towards lower albumin levels in the older age groups. This decrease in serum albumin is greater for White males (3.37 vs 4.49) than in Black females (3.74 to 4.47 g/100ml). The male albumin levels are 0.7-0.1 g/100ml greater than females between the ages of 11 and 34, after which age the reverse is true of the male getting slightly down the female slope and the two sexes become very similar. Finally, in the small groups aged 75 and older (17 males, 13 females), the male value falls below the female mean value.

Serum uric acid. Uric acid data is presented as for serum protein in tabular and graphic form (Table 12 and Figure 2).

Table 12

Serum Uric Acid by Race and Sex

Group	n	*Mean Uric Acid
1,340 Whites (both sexes)	1,255	5.48 ± 1.52
605 White males	575	6.37 ± 1.36
735 White females	680	4.72 ± 1.20
85 Negroes (both sexes)	80	5.16 ± 1.54
31 Negro males	28	6.05 ± 1.31
54 Negro females	52	4.68 ± 1.44
39 Negroes aged 21-34 years	36	4.58 ± 1.49
46 Negroes aged 35-76 years	44	5.63 ± 1.42

*Units in mg/100ml ± 1 S.D.

These data clearly show the sex difference of males greater than females which is similar in both races, but a difference somewhat greater in the Whites (1.65 vs. 1.37 mg/100ml). There is a racial difference of only 0.3 mg/100ml greater in Whites which is present in males or in the combined sexes group, but absent when females of the two races are compared. An increase in serum uric acid is present with increasing age in White males (5.83 to 7.06 mg/100ml), White females (4.45 to 5.14 mg/100ml) and Negroes when compared young vs. old (4.58 to 5.63 mg/100ml).

Serum urate data. Urate acid data is presented as for serum protein in tabular and graphic form (Table 12 and Figure 2).

Table 12

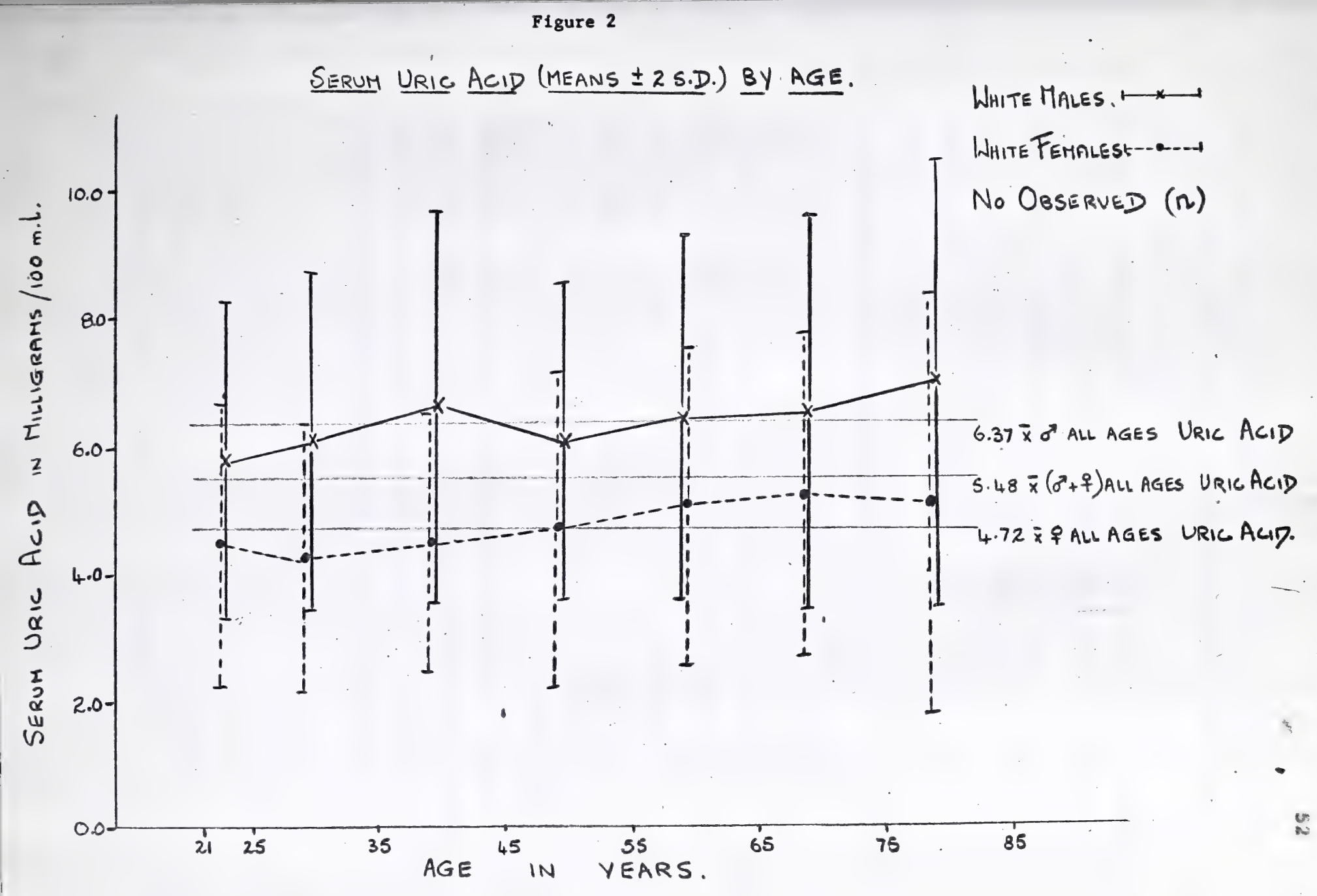
Serum Urate Acid by Race and Sex

Group	n	*Mean Urate Acid
1,349 Whites (Both sexes)	1,351	5.48 ± 1.38
805 White males	575	6.37 ± 1.36
785 White females	680	4.72 ± 1.20
85 Negroes (both sexes)	80	5.16 ± 1.34
31 Negro males	28	6.05 ± 1.31
54 Negro females	52	4.42 ± 1.04
19 Negroes aged 21-34 years	16	4.38 ± 1.49
46 Negroes aged 35-76 years	44	5.63 ± 1.42

*Units in mg/100ml ± 1 S.D.

These data clearly show the sex difference of males greater than females which is similar in both cases, but a difference important greater in the White (left vs. 1.31 mg/100ml). There is a racial difference of only 0.3 mg/100ml greater in White males than in the two cases are compared. In females in serum urate acid is greater with increasing age in White males (1.23 to 1.38 mg/100ml), White females (0.42 to 0.34 mg/100ml) and Negroes when compared young vs. old (1.38 vs 1.42 mg/100ml

Figure 2


SERUM URIC ACID (MEANS ± 2 S.D.) BY AGE.
WHITE MALES.
WHITE FEMALES
No OBSERVED (n)
SERUM URIC ACID IN MILLIGRAMS/100 m.L.
10.0
8.0
6.0
4.0
2.0
0.0
6.37 x̄ ♂ ALL AGES URIC ACID
5.48 x̄ (♂+♀) ALL AGES URIC ACID
4.72 x̄ ♀ ALL AGES URIC ACID.
21
25
35
45
55
65
76
85
AGE IN YEARS.
(n) MALES: 28 93 119 160 98 58 18
(n) FEMALES: 39 108 146 182 117 73 15

Among Whites this increase in males (1.23) is 78% greater than that in females (0.69). The uric acid increase with age among White females follows a smooth and gradual increase when compared with that in males. In males starting at 5.83 mg/100ml at age 21-24, there is a continuous rise until an early peak (6.64 mg/100ml) at age 35-44 with a fall by age 45-54 to the earlier levels, then a lesser rise by age 55-64 (6.45 mg/100ml), a plateau until 65-79 (6.58 mg/100ml) and a final rise again amongst the 18 over 75 (7.06 mg/100ml).

Paper electrophoresis results

Total globulins and globulin fractions. Before presenting and describing the results of paper electrophoresis studies on individual globulin fractions (alpha-1, alpha-2, beta and gamma globulins), a summary table is presented with mean values for each race and sex, as well as for young vs. older Negroes. Electrophoresis was completed on the sera of 973 Whites and 56 Negroes. For additional reference, the summation of the four globulin fraction means is included as "total globulins". It should be stressed that the total globulins figure was not, in fact, determined by electrophoretic techniques, but rather is defined as the difference between the total protein and albumin values obtained by the AutoAnalyzer. The g/100ml values for the individual globulin fractions were obtained by proportioning this "total globulins" among the four fractions according to their relative amounts as determined by the electrophoresis.

Table 13

Paper Electrophoresis of Serum Globulins
(g/100ml ± 1 S.D.)

Group	n	alpha$_1$	alpha$_2$	beta	gamma	total globulins
1,340 Whites (both sexes)	973	0.305 ±0.122	0.499 ±0.181	0.681 ±0.228	0.897 ±0.348	2.382
605 White males	438	0.297 ±0.130	0.475 ±0.184	0.668 ±0.230	0.872 ±0.344	2.312
735 White females	535	0.312 ±0.114	0.518 ±0.177	0.692 ±0.227	0.918 ±0.351	2.440
85 Negroes (both sexes)	56	0.326 ±0.159	0.534 ±0.181	0.721 ±0.218	1.078 ±0.431	2.659
31 Negro males	18	0.315 ±0.105	0.473 ±0.157	0.651 ±0.175	1.007 ±0.391	2.446
54 Negro females	38	0.331 ±0.181	0.563 ±0.186	0.755 ±0.230	1.112 ±0.449	2.761
39 Negroes aged 21-34 years	23	0.347 ±0.225	0.538 ±0.203	0.758 ±0.258	1.058 ±0.508	2.701
46 Negroes aged 35-76 years	33	0.310 ±0.092	0.531 ±0.166	0.696 ±0.185	1.092 ±0.375	2.629

Table 13

Paper Electrophoresis of Serum Globulins
($\bar{x}$/100ml ± 1 S.D.)

Group	n	alpha$_1$	alpha$_2$	beta	gamma	Total globulins
1,340 Whites (born oversea)	973	0.305 ±0.122	0.499 ±0.181	0.681 ±0.224	0.897 ±0.344	2.382
815 White males						
		±0.130	±0.164	±0.230	±0.344	
745 White females						
		±0.114	±0.177	±0.227	±0.371	
63 Negroes (Dark skins)	36					
		±0.188	±0.184	±0.218	±0.418	
31 Negro males	18	0.315	0.475	0.651	1.007	2.448
		±0.105	±0.157	±0.175	±0.341	
54 Negro females	38	0.331	0.563	0.755	1.112	2.761
		±0.181	±0.166	±0.226	±0.449	
39 Negroes	23	0.347	0.518	0.758	1.038	2.701
aged 21-49 years		±0.121	±0.[illegible]	±0.[illegible]	±0.[illegible]	
14 Negroes	13	0.318			1.002	2.627
aged 25-74 years		±0.093	±0.164	±0.[illegible]	±0.[illegible]	

Alpha-1 globulin (Figure 3). Differences from the mean alpha-1 globulin level (0.305 ± 0.122 g/100ml) for the entire White sample are only minimal when sexes are separated, those for White males (0.297 ± 0.130 g/100ml) being somewhat lower than for White females (0.312 ± 0.114 g/100ml). Negroes as a group show higher mean (0.326 ± 0.159 g/100ml) than Whites, and as in Whites, Negro males (0.315 ± 0.105 g/100ml) have mean values lower than those for Negro females (0.331 ± 0.181 g/100ml).

Examination of the age-specific curves in Figure 3 indicates no significant change with age in the alpha-1 globulin for either sex until after ages 55-64 when male levels rise above female levels to 0.342 ± 0.153 g/100ml at 65-74, and 0.358 ± 0.107 g/100m; at 75 and older. Female Whites show a final high with a rise to 0.352 ± 0.123 g/100ml at 75 and older. The Negroes when divided by age show a fall from 0.347 ± 0.225 g/100ml in the younger group, to 0.310 ± 0.092 g/100ml in the group aged 35 years or more.

Alpha-2 globulin (Figure 4). Mean alpha-2 globulin for White males and females combined is 0.499 ± 0.181 g/100ml, with higher levels in females (0.518 ± 0.177 g/100ml) than in males (0.475 ± 0.184 g/100ml). This sex difference is greatest in the 21-24 year old group (females, 0.533 ± 0.181 g/100ml; males, 0.418 ± 0.208 g/100ml), and is reversed only in the 75 and older group (females, 0.585 ± 0.163 g/100ml; males, 0.624 ± 0.148 g/100ml). There is a relative plateau in both sexes between the ages of 25 and 54 years, with both sexes increasing after 54 years in each subsequent age group. This increase is more rapid in White males than in White females.

In comparing the two races, Negroes have a higher mean (0.534 ± 0.181 g/100ml) for combined sexes which is a result of the markedly

Figure 3

ALPHA 1 GLOBULIN

(MEANS ± 2 S.D.) BY AGE.

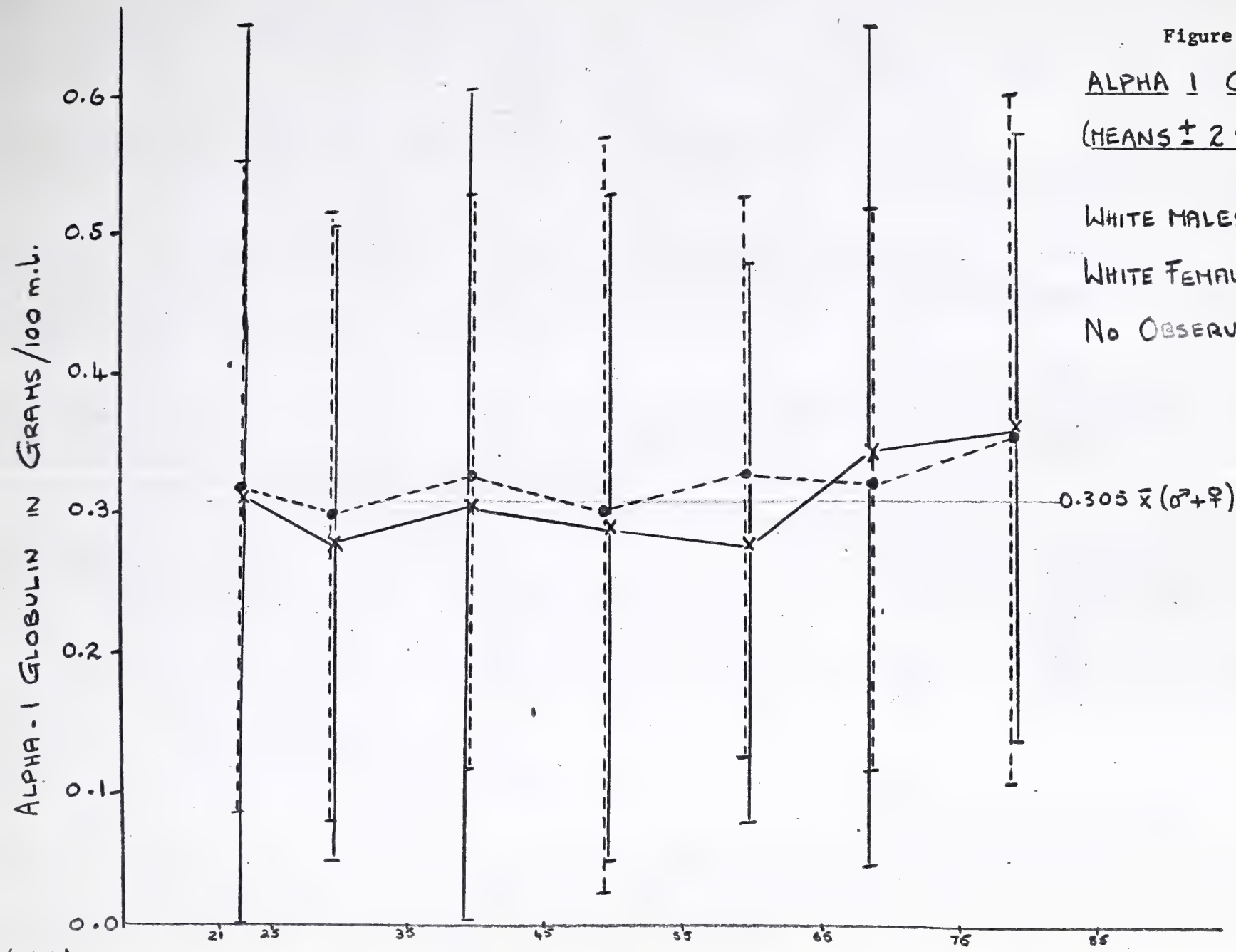
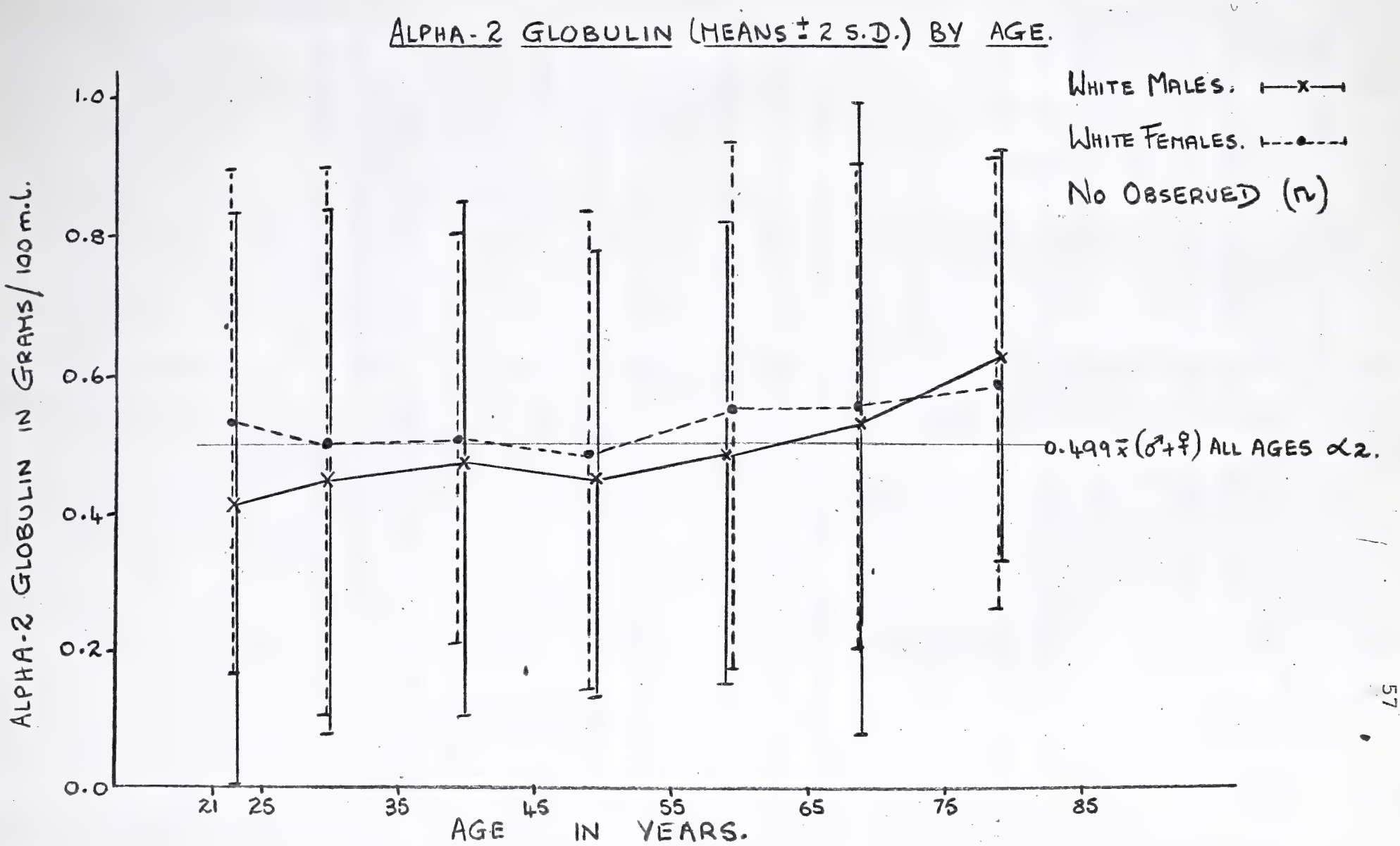


Figure 4



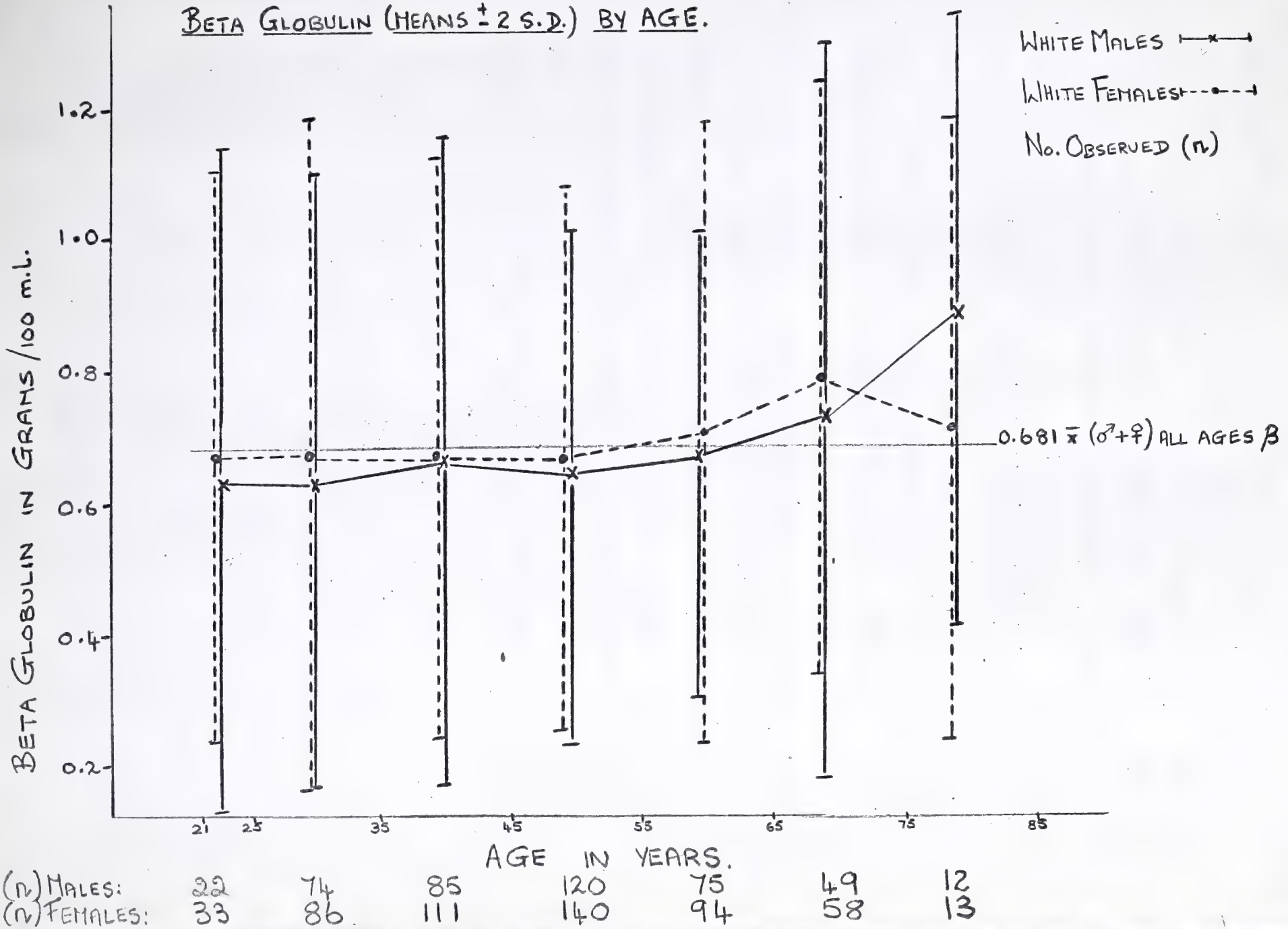
(n) MALES.	22	74	85	120	75	49	12
(n) FEMALES.	33	86	111	140	94	58	13

higher mean in Negro females (0.563 ± 0.186 g/100ml). There is no difference in the mean in Negro males (0.473 ± 0.157 g/100ml) from that of White males, and there is little difference in the means of young Negroes (0.538 ± 0.203 g/100ml) as compared to older Negroes (0.531 ± 0.166 g/100ml).

Beta globulin (Figure 5). Little sex difference is found in mean beta globulin levels among Whites when comparing the combined sexes (0.681 ± 0.228 g/100ml) with either the males (0.668 ± 0.230 g/100ml) or the females (0.692 ± 0.229 g/100ml) separately. There is essentially no change with age in either sex until after ages 45-54 when both males and females show an increase in beta globulins. The rate of increase is similar in both sexes until ages 65-74, after which the males continue an increasingly rapid rise (0.880 ± 0.234 g/100ml) to surpass the female level which has fallen from 0.786 ± 0.226 g/100ml at ages 65-74 to 0.714 ± 0.235 g/100ml at the oldest ages.

As with the alpha-2 globulins, a comparison of the two races shows a higher mean beta globulin level among Negroes (0.721 ± 0.218 g/100ml) which is due to the high mean in Negro females (0.755 ± 0.230 g/100ml). Negro males show a markedly lower mean (0.651 ± 0.175 g/100ml) than the Negro females, and one which is also definitely lower than the mean for White males. Among the broad age groups in Negroes, as with alpha-1 globulin the younger show a higher mean beta globulin (0.758 ± 0.258 g/100ml) than do the group aged 35 and over (0.696 ± 0.185 g/100ml).

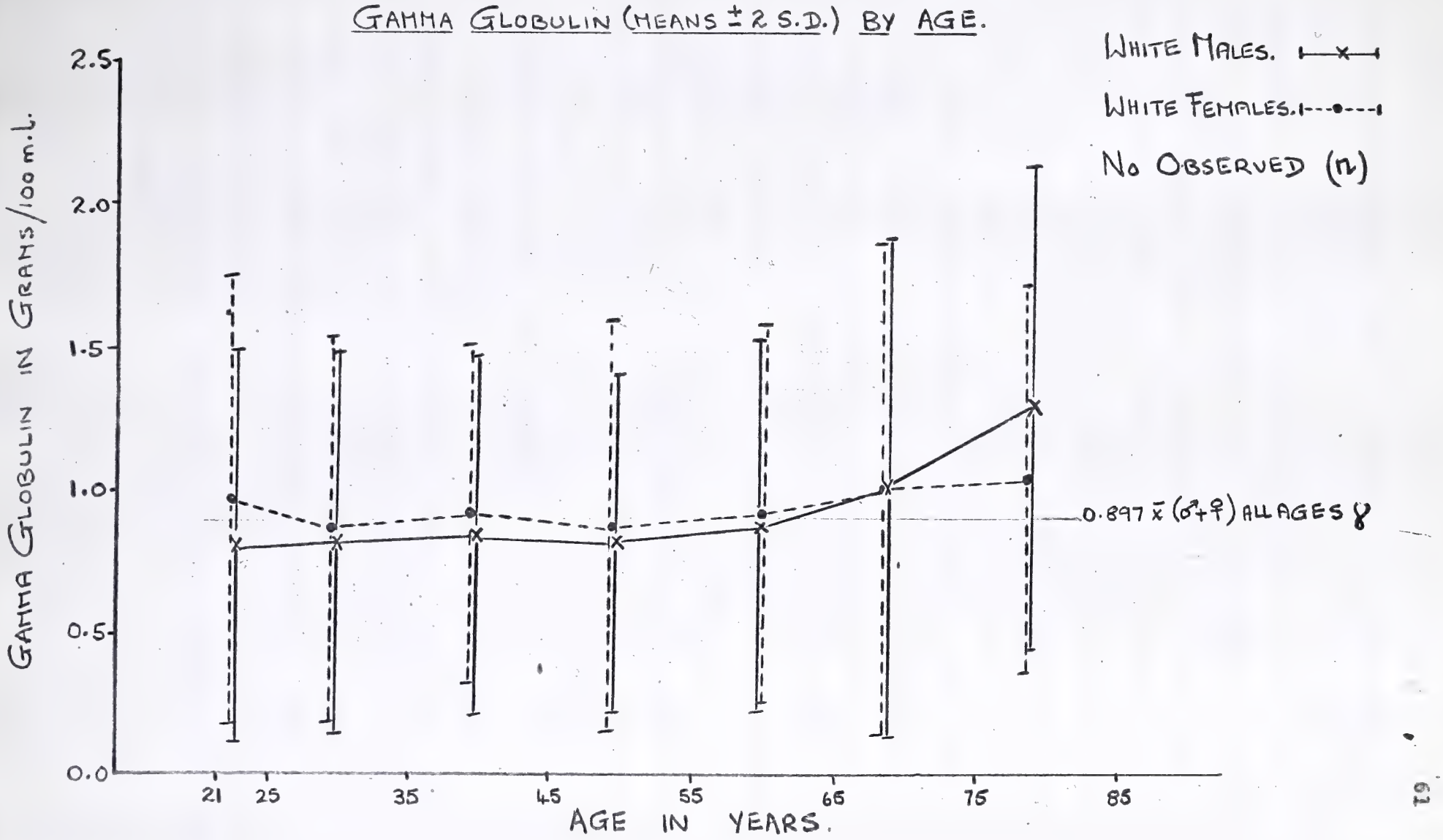
Figure 5


BETA GLOBULIN (MEANS ± 2 S.D.) BY AGE.
WHITE MALES
WHITE FEMALES
No. OBSERVED (n)
BETA GLOBULIN IN GRAMS/100 m.L.
1.2
1.0
0.8
0.6
0.4
0.2
0.681 x̄ (♂+♀) ALL AGES β
21
25
35
45
55
65
75
85
AGE IN YEARS.
(n) MALES: 22 74 85 120 75 49 12
(n) FEMALES: 33 86 111 140 94 58 13

Gamma globulin (Figure 6). Mean gamma globulin level in Whites is 0.897 ± 0.348 g/100ml for the combined sexes, 0.872 ± 0.348 g/100ml in males and 0.918 ± 0.351 g/100ml in females. The excess in gamma globulins in females is greatest at ages 21-24 (0.976 ± 0.392 g/100ml vs. 0.805 ± 0.347 g/100ml in males) and gradually diminishes to -0.001 at ages 65-74 (female mean of 1.020 ± 0.424 g/100ml) and then is reversed after age 74 when male means of 1.032 ± 0.414 g/100ml greatly exceed female means of 1.052 ± 0.336 g/100ml. However, the samples are small after age 74. There is relatively little change with age for White males between 21 and 54, nor for White females between 25 and 64 years.

Negroes have a higher gamma globulin level than Whites when comparing sexes combined (Negroes: 1.078 ± 0.431 g/100ml), males (Negroes: 1.007 ± 0.391 g/100ml) or females (Negroes: 1.112 ± 0.449 g/100ml). The excess in mean gamma globulin in females is 10.4% in Negroes compared with 4.1% excess in White females, with respect to levels in males of the same race. There is a small rise with age among the Negroes, with the mean of 1.058 ± 0.508 g/100ml in the younger group (mean age 27.3 years) rising to 1.092 ± 0.375 g/100ml in the older group (mean age 47.0 years).

Figure 6



(n) MALES:	22	74	85	120	75	49	12
(n) FEMALES:	33	86	111	140	94	58	13

Patterns of serum protein fractions (Figures 1, 7, 8 and Tables 11, and 13). As mentioned earlier, the findings for mean total protein and albumin by race and sex are that total protein varies little, being highest in White males and Negro females. At the same time albumins show higher levels in Whites and in males within each racial group. These differences produce, by subtraction, higher total globulins in females, with those in the Negro females being clearly above all others.

In examining the globulins for each fraction, Negroes have higher values than Whites, females have higher values than males of the same race, and Negro female levels are higher than those in White females. Negro males have higher alpha-1 and gamma globulins when compared with White males, but have alpha-2 levels just below those of White males and beta globulins rather lower than White males. Whites differ by sex mostly in the alpha-2 and gamma fractions, while Negroes show large differences in all but the alpha-1 fraction. In differences between the races, the least is in the alpha-1 globulin fraction, more in the alpha-2 and beta fractions (which are mainly accounted for by the high levels in Negro females) and clearly the most racial difference (notable in each sex) lies in the gamma globulins.

When considering age patterns in serum globulin fractions, the findings in Whites are that after various changes in the third decade of life, levels remain relatively stable between ages 25 and 54 years. After 54 there is a rise in each fraction in both sexes, and this increase is consistently more rapid in males. Furthermore, although females have higher globulin levels at most ages, after 74 male levels in each fraction are greater. In alpha-1 and gamma globulin this reversal takes place after 64.

albumin by race and sex are that total protein varies little, being
lowest in White males and highest in Negro females. In the same
race, males have higher levels in Whites and in males within each racial group.
These differences produce, by subtraction, higher total globulins in
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values than Whites, females have higher values than males of the same
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and beta globulins rather lower than White males. Whites differ by
differences in all but the alpha-1 fraction. In difference between
the races, the lowest is in the alpha-1 globulin fraction, more in the
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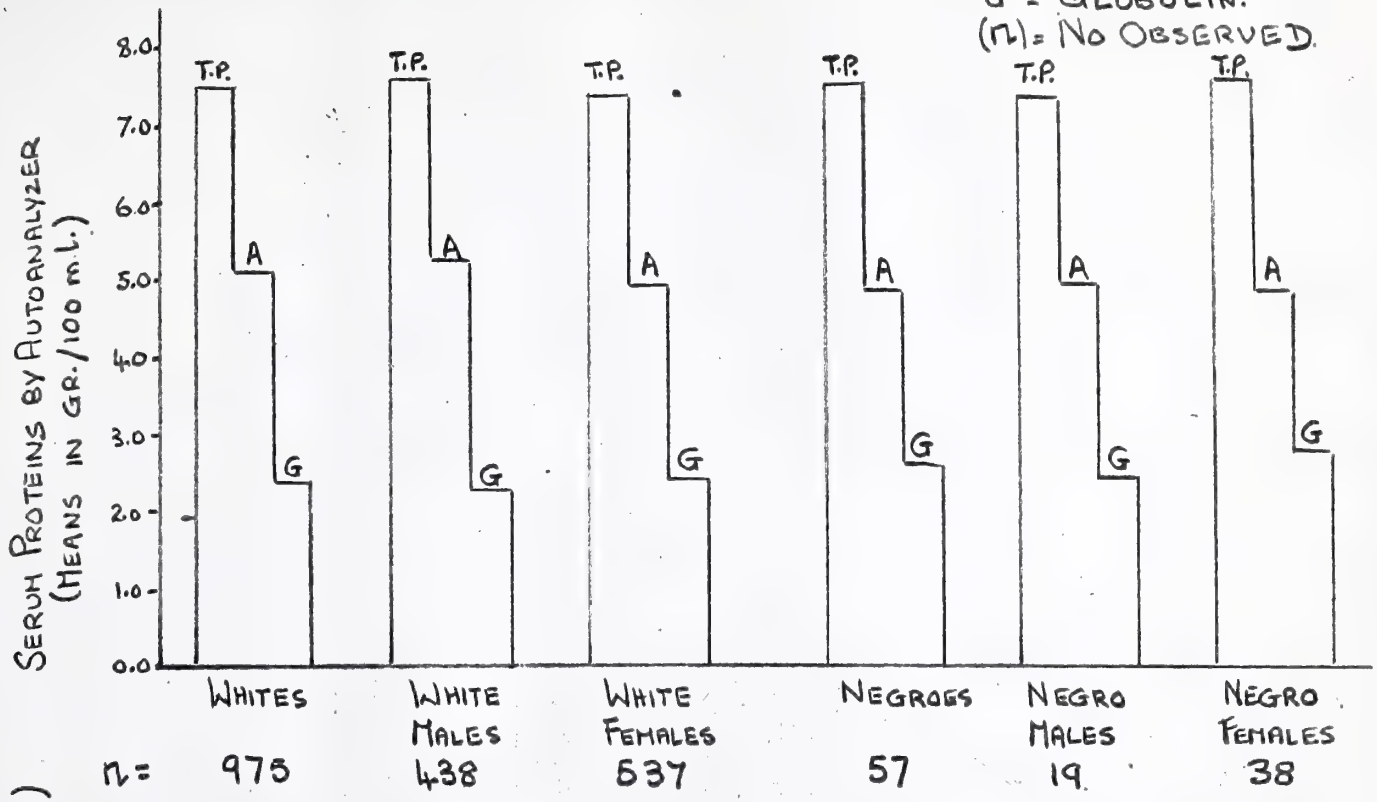
When considering age patterns in serum globulin fractions, the
findings in Tables are that sex differences in the total levels
of life, levels remain relatively stable between ages 25 and 54 years.
After 54 there is a rise in each fraction in both sexes, and this in-
crease is consistently more rapid in males. Furthermore, although
females were higher globulin levels at most ages, after 74 only [illegible]
increase fractions are greater. In alpha-1 and gamma globulin this reversal
takes place after 64.

Figure 7

Serum Protein Mean Values By Race and Sex.

T.P. = Total Protein.
A = Albumin.
G = Globulin.
(n) = No Observed.



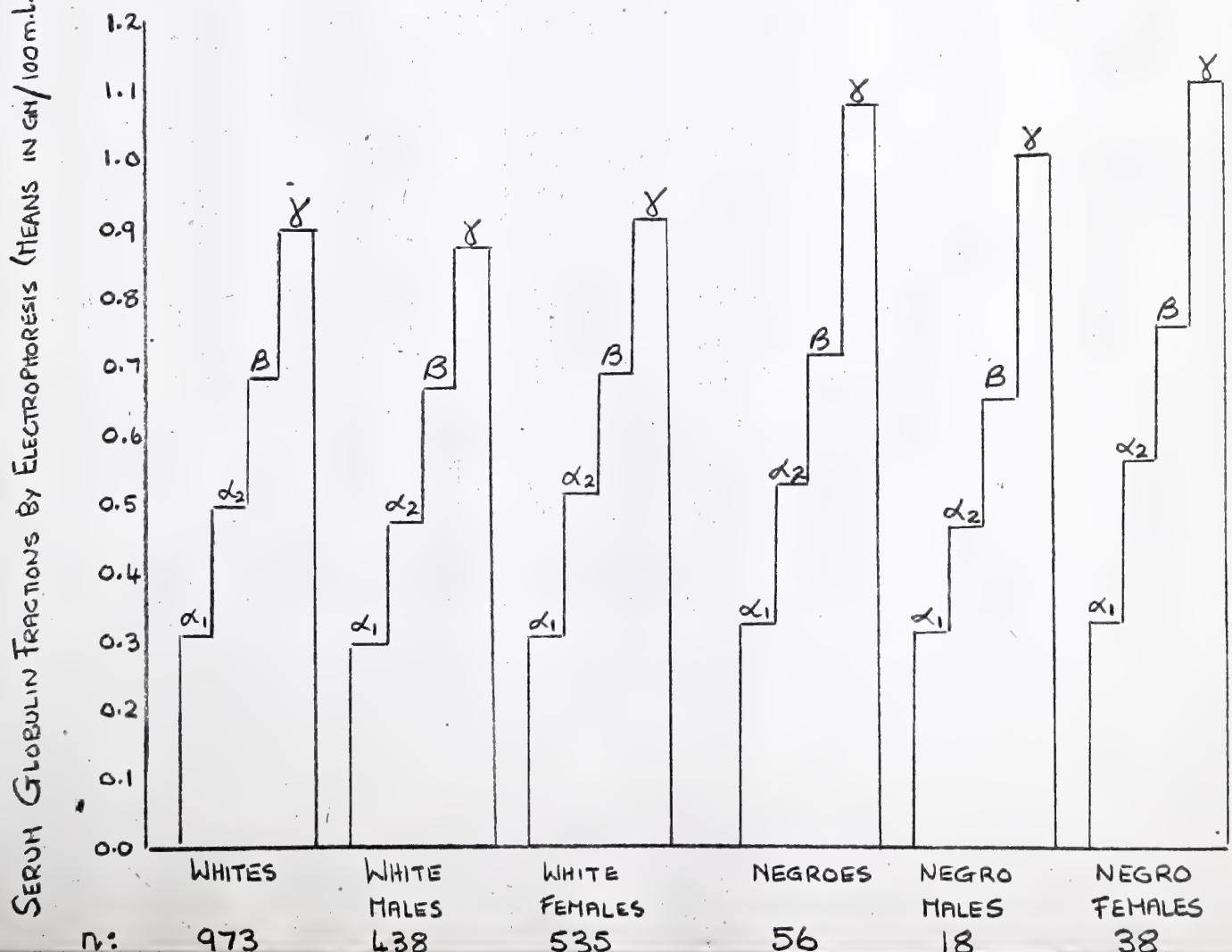
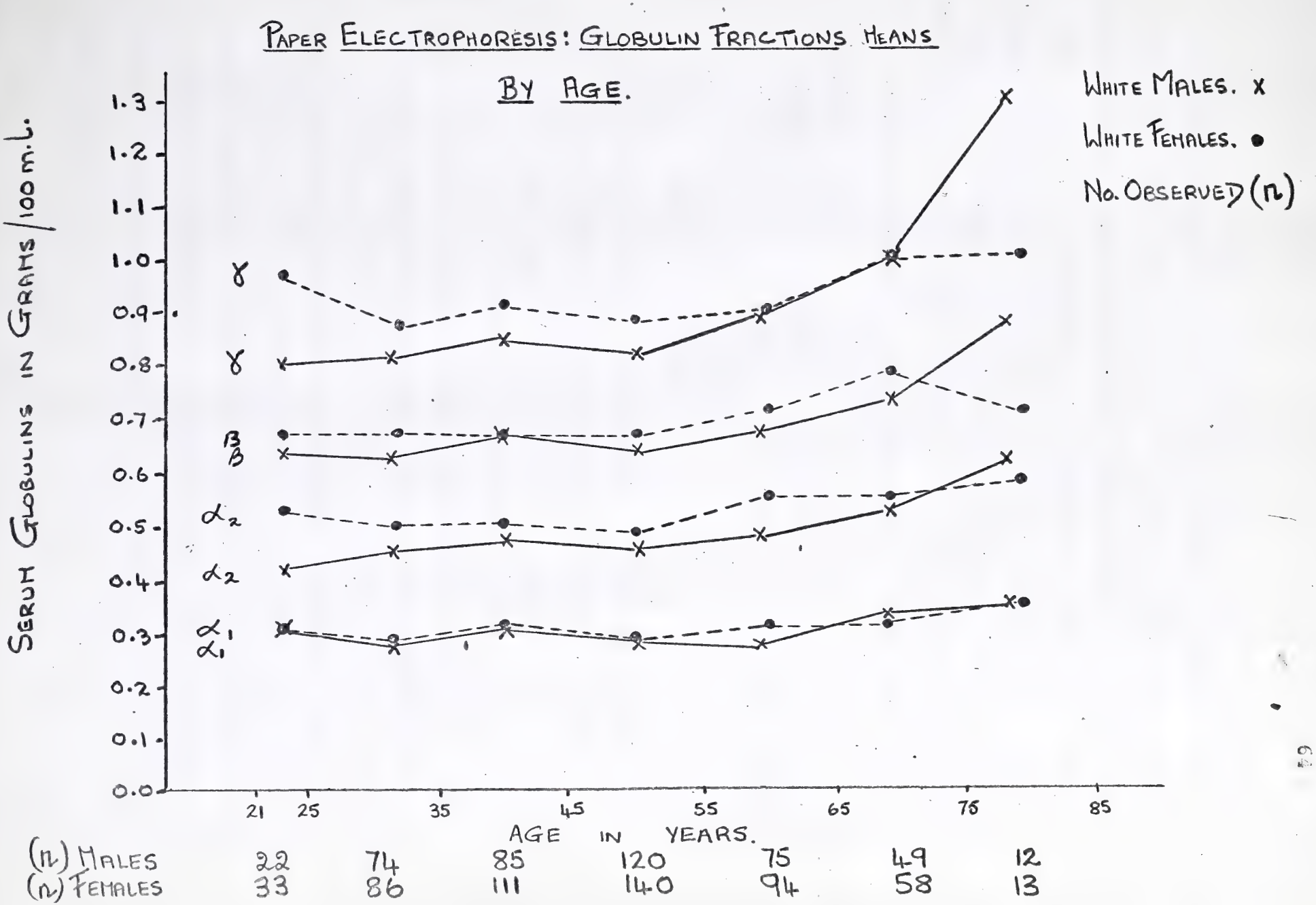


Figure 8


PAPER ELECTROPHORESIS: GLOBULIN FRACTIONS MEANS
BY AGE.
SERUM GLOBULINS IN GRAMS / 100 M.L.
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
γ
γ
β
β
α2
α2
α1
α1
21
25
35
45
55
65
75
85
AGE IN YEARS.
WHITE MALES. x
WHITE FEMALES. ●
No. OBSERVED (n)
(n) MALES 22 74 85 120 75 49 12
(n) FEMALES 33 86 111 140 94 58 13

The absolute change with age appears to be in reverse order to the electrophoretic mobilities of the various fractions. That is, alpha-1 (the fastest moving fraction) varies least with age, alpha-2 next, beta more and gamma globulin most of all. As an approximate estimate of whether this difference in absolute change is a result of the quantity of each fraction usually present in serum, a few simple calculations are presented. If the difference in mean values of the youngest and the oldest age groups for each fraction in White males and White females is expressed as a percentage of the mean in the youngest group, the increases with age are as follows:

		males	females
1)	alpha-1	14.7%	11.0%
2)	alpha-2	49.6%	9.8%
3)	beta	38.4%	5.9%
4)	gamma	61.7%	7.8%

As these simple differences between means in the extreme age groups (which are small samples) do not represent the entire age patterns, they must be taken only as general trends. For example, the mean gamma globulin decreases in females after ages 21-24 to a plateau until after age 64. If the baseline gamma globulin in females is taken as that at ages 25-34, the increase by the oldest age is 19.9%. Similarly, the change in beta globulin in females from ages 21-24 to 65-74 is 16.8%. We may therefore say that there is a difference in both the absolute and the relative increases with age in the various globulin fractions, and that the trend is more striking in males than in females. Depending upon the age limits examined, the relative changes among globulin fractions in females are rather similar or are more marked in the beta and gamma fractions.

Among Negroes, the change within the two age categories studied show a more variable pattern. There are minor differences in total protein, albumin and, therefore, total globulins by age, but the alpha-1 and beta fractions (and the alpha-2 globulins minimally) decrease with age while the gamma globulins increase with age.

Hemoglobin results. Mean hemoglobin levels are higher in males compared with females of the same race, and higher in Whites when compared with Negroes of the same sex. These mean values are given in Table 14 below.

Table 14

Hemoglobin (g/100ml) by Race and Sex

	1,340 Whites	605 White males	735 White	85 Negroes	31 Negro males	54 Negro females
n	1,184	539	645	74	25	49
mean	14.46	15.44	13.64	13.40	14.64	12.76
1 S.D.	±1.63	±1.34	±1.38	±1.94	±1.82	±1.68

Examining the age curves for Whites in Figure 9, it can be seen that in males there is no change until ages 45-54 when the hemoglobin falls progressively from a mean of 15.48± 1.48 g/100ml to a mean after age 74 of 14.95 ± 1.20 g/100ml. The pattern in White females is different, starting at ages 21-24 at 13.67 ± 1.19 g/100ml, falling to a low of 13.19 ± 1.28 g/100ml at ages 25-34, rising to 13.82 ± 1.53 g/100ml between ages 45-54, and then remaining within 0.09 above that level for the remaining ages.

Negroe hemoglobin levels rise with age from 12.98 ± 2.06 g/100ml for the 21-34 year old group, to 13.75 ± 1.77 g/100ml for the group aged 35 and above.

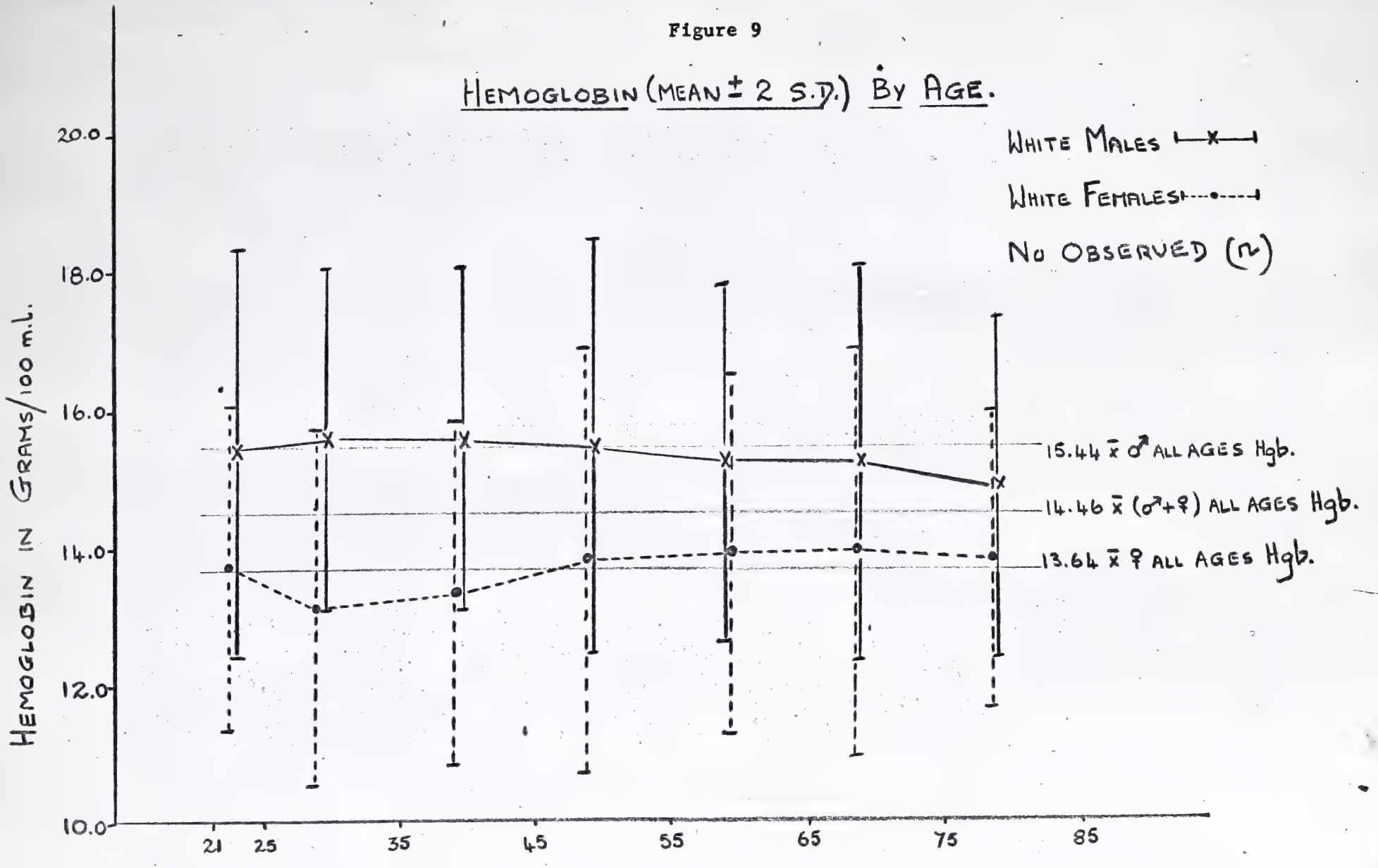
Table 14

Hemoglobin (g/100ml) by Race and Sex

n	1,164	579	645	74	25	49
mean	14.66	13.96	13.44	13.40	14.24	13.34
1 s.d.	±1.83	±1.44	±1.39	±1.49	±1.81	±1.44

Figure 9

HEMOGLOBIN (MEAN ± 2 S.D.) BY AGE.



(n) MALES:	28	81	112	151	89	60	17
(n) FEMALES:	37	100	137	176	113	67	15

Laboratory Data Correlations with Social Class and Age

Social class with total protein, protein fractions and uric acid. Correlation coefficients (r) between the various factors studied were done in order to gain some indication of the extent and direction of the relationship between these factors, both in the case of relationships previously reported in the literature and of those which became apparent through the analysis of means when groups were sorted in manner described in the present study. Furthermore, the ease with which great numbers of correlation coefficients are generated by use of computers offers one means of uncovering clues to relationships within large quantities of data which otherwise are unsuspected.

Correlation coefficients between social class and total protein, albumin, globulin fractions and uric acid were done, and in Whites of all ages the only significant correlations with social class are those of serum albumin in an inverse relationship (combined sexes, $r=-0.0824$, $n=965$, $p<0.01$; males, $r=-0.1156$, $n=432$, $p<0.05$; females, $r=-0.0434$, $n=533$, p-NS at 5% level), and of serum uric acid in White females in a direct relationship ($r=0.1201$, $n=676$, $p<0.01$). That is, among Whites albumins tend to be greater in the higher socioeconomic groups except for females studied alone, and among females, uric acids are higher in the lower socioeconomic groups.

When age subgroups are considered, several correlations are significant at the 5% level for total protein, albumin and hemoglobin in the same inverse relationship noted above for albumin, but the pattern is sporadic. For uric acid and globulin fractions, correlations of significant level with social class are also sporadic among the age subgroups,

Secondary Data Correlations with Social Class and Age

Social class with total protein, albumin fraction and urea

Correlation coefficients (r) between the various factors studied were done in order to gain some indication of the extent and direction of the relationship between these factors, both in the case of relationships previously reported in the literature and of those which became apparent through the analysis of social class mental ratio. Particularly in the present study. Furthermore, the same study which first examined of interrelations were examined by use of computers although some of interrelations could be relationship within large quantities of data which otherwise are unsuspected.

Correlation coefficients between social class and total protein, albumin, globulin fractions and urea were done, and to obtain of all ages the only significant correlations with social class are those of serum albumin in an inverse relationship (combined sexes, r=-0.0824, p<0.01 males, r=-0.1211, males, p<0.01; females, r=-0.0314, p=.02, p=.06 at 99 level) and of serum urea with in which females (a direct relationship (r=0.1201, n=576, p<0.01). That is, among white although urea tend to be greater in the higher socioeconomic groups for females studied alone, and among females, with white in the lower socioeconomic groups.

When age subgroups are considered, separate correlations are significant at the 5% level for total protein, albumin and homogenin in the same inverse relationship noted above for albumin, but the pattern is quarterly. For white and Spanish females, correlations of signi-

but values of r are neither consistently positive nor consistently negative.

No significant correlations with social class are present in any of the Negro groups.

Age with total protein, protein fractions and uric acid. If correlations with age are examined for each of the variables under consideration, significant levels are obtained in Whites in each instance except alpha-1 globulin for at least two of the three groupings of combined sexes, males or females, although some of the correlations are positive and others are negative. This fact is shown below in Table 15. When the more narrow age subgroups of ten years (or four years in the case of the 21-24 year old subgroup), significant levels of correlation are expectedly few and sporadic.

Among Negroes, age and hemoglobin correlate significantly and positively in the combined sexes ($r=0.2970$, $n=72$, $p=0.01$) and in females ($r=0.3649$, $n=49$, $p<0.01$), but not in males. Age and uric acid correlate significantly and positively in each of the three Negro groups: combined sexes, $r=0.4579$, $n=80$, $p<0.001$; males, $r=0.4253$, $n=28$, $p<0.05$; and females, $r=0.4363$, $n=52$ $p<0.01$. When the two broad Negro age subgroups (21-34 and 35-76 years) are examined, age shows a negative correlation in the younger group with alpha-2 globulin ($r=0.4656$, $n=23$) and a positive correlation with hemoglobulin ($r=0.4121$, $n=34$) and with uric acid in both the younger ($r=0.3333$, $n=36$) and older ($r=0.3626$, $n=44$) groups, all significant at the 5% level.

but values of x are neither unanimously positive nor consistently negative.

The significant correlations with social class are present in any of the three groups.

Age with cereal protein, protein fractions and uric acid. If correlations with age are examined for each of the variables under investigation, significant levels are obtained in eleven instances (see Table 14). Statistically two at least one of the three groupings of combined sexes, males or females, although some of the correlations are positive and others are negative. This fact is shown below in Table 15. When the more severe age subgroups at two years (or four years in the case of the 11-24 year old subgroup), significant levels of interrelations are experienced few and sporadic.

Among degrees, age and hemoglobin correlate significantly and positively in the combined sexes ($r=0.3649$, $n=49$, $p<0.01$), but not in males. Age and uric acid correlate significantly and positively in each of the three groups (combined sexes, $r=0.4579$, $n=80$, $p<0.001$; males, $r=0.4253$, $n=28$, $p<0.05$; and females, $r=0.4181$, $n=52$ $p<0.01$. When the two broad height age subgroups (13-14 and 15-18 years) are examined, age shows a negative correlation in the younger group with alpha-2 globulin ($r=-.4831$, $n=17$) and a positive correlation with hemoglobin ($r=0.4431$, $n=17$) and a positive correlation with hemoglobin ($r=0.4431$, $n=17$) and also in both the younger ($r=.111$, $n=19$) and older ($r=.2519$, $n=31$) groups, all significant at the 5% level.

Table 15

Correlations between Age and Laboratory Findings in Whites

	White Males			White Females			White Sexes Combined		
Age and	n	r	p	n	r	p	n	r	p
Total Protein	-	-	NS	537	-0.0871	<0.05	974	-0.0784	<0.05
Albumin	437	-0.3617	<0.001	537	-0.2385	<0.01	974	-0.2811	<0.001
Alpha-1 Globulin	-	-	NS	-	-	NS	972	0.0690	<0.05
Alpha-2 Globulin	437	0.1635	<0.01	535	0.0976	<0.05	972	0.1251	<0.01
Beta Globulin	437	0.1547	<0.01	535	0.1273	<0.01	972	0.1387	<0.01
Gamma Globulin	437	0.2039	<0.01	-	-	NS	972	0.1239	<0.01
Uric Acid	574	0.0959	<0.05	680	0.2716	<0.001	1254	0.1655	<0.01
Hemoglobin	538	-0.1096	<0.05	645	0.1645	<0.01	-	-	NS

Table 13

Correlations between Age and Laboratory Findings
in Whites

Intercorrelations between Total Protein, Albumin, Globulin Fractions, Uric Acid and Hemoglobin

Total protein. As might be expected, total protein in Whites correlates significantly and positively to a very high degree with albumin and each of the four globulin fractions, whether sexes are considered separately or combined. These correlations are shown in Table 16 for groups with ages combined. When age subgroups are considered, similar values of r are found consistently. The range of values of correlation coefficients with total proteins in the age subgroups in Whites are as follows:

Males, albumin 0.1518-0.5057, globulins 0.3255-0.8342;

Females, albumin 0.1868-0.4865, globulins 0.2701-6982;

Combined Sex, albumin 0.1341-0.4867, globulins 0.3135-0.7077.

The degree of correlations shows no pattern with age. A single negative value of r (-0.1289) for total protein and albumin in White males aged 21-24 is not included in the ranges listed, and is probably due to sampling. Two other points can be noted. First, correlations for total protein and the four globulin fractions in any given group or age subgroup studied show rather consistently an increase in r as one proceeds from alpha-1 through alpha-2, beta and gamma globulins. That is, at any given age total protein correlates more highly with the gamma fraction than with any other globulin fraction. Secondly, of the few age subgroups in which this pattern of correlation with total protein alters somewhat, ages 55-64 in males, females and combined sexes show a similar decrease in r from alpha-1 to alpha-2 before continuing on to a maximum value in the gamma fraction. Nevertheless, these values of r for total protein and alpha-2 globulin still maintain high levels of significance.

Total protein. As might be expected, total protein is quite correlative
significantly and positively in a very high degree with albumin and each
of the four globulin fractions, whether sexes are considered separately
or combined. These correlations are shown in Table 13 for young white
ages combined. When age subgroups are considered, similar values of r
are found consistently. The range of values of correlation coefficients
with total protein in the age subgroups in Whites are as follows:

Males, albumin [illegible], globulins [illegible];

Females, albumin [illegible], globulins [illegible];

Combined Sex, albumin 0.1[illegible]-0.4881, globulins 0.1313-0.3617.

The figure of [illegible] shows by patients with age. A single comparison
value of r = [illegible] for total protein and albumin in White males aged
21-24 is not included in the ranges listed, and is probably due to
sampling. The other points can be noted. First, correlations for total
protein and the four globulin fractions in any given group or age sub-
group studied show rather consistently an increase in r as one proceeds
from alpha-1 through alpha-2, beta and gamma globulins. That is, at
any given age total protein correlates more highly with the gamma fraction
than with any other globulin fraction. Secondly, at the five age sub-
groups in which this pattern of correlation with total protein albumin
somewhere, save in [illegible] females and combined sexes [illegible],
surpasses in r from alpha-1 before vanishing at 50 + females
value in the gamma fraction. Nevertheless, these values at r for total

Table 16

Correlation Coefficients (r) and Sample Size (n)
Between Total Protein and Protein Fractions

Total Protein &:	Albumin	Globulins			
		Alpha-1	Alpha-2	Beta	Gamma
All Whites	0.3426 (975)	0.4436 (973)	0.4551 (973)	0.5541 (973)	0.6064 (973)
White Males	0.3095 (438)	0.4597 (438)	0.5187 (438)	0.5789 (438)	0.6149 (438)
White Females	0.3435 (537)	0.4494 (535)	0.4360 (535)	0.5512 (535)	0.6210 (535)
All Negroes	0.1444 (57)	0.4417 (56)	0.5497 (56)	0.5957 (56)	0.7634 (56)
Negro Males	0.1291 (19)	-0.4045 (18)	0.7901 (18)	0.7307 (18)	0.7024 (18)
Negro Females	0.1682 (38)	0.6143 (38)	0.4665 (38)	0.5493 (38)	0.7792 (38)

Table 13

Correlation Coefficients (r) and Table Size (N)
Between Total Female and Female Prosecutions

	(Combine				
Total Female P.	(Illegit.	(Liquo-	(Liquo-	Vice	Combi
All Offense					
Other Offen					
Petty Female					
All Theft					
Simple Assa					
Other Female					

Negroes of either sex do not show a significant correlation between total protein and albumin, but do show positive correlations significant at the 1.0-0.1% level between total protein and the four globulin fractions when studied by either sex or age. The only exception to this is between total protein and alpha-1 globulin for which the correlation is not significant in two instances, a negative value in Negro males ($r=-0.4045$, $n=18$) and a positive r in Negroes aged 35-76 ($r=0.1754$, $n=33$).

Correlations between total protein and uric acid are positive and significant in Whites for the combined sexes ($r=0.1571$, $n=969$, $p<0.01$), the males ($r=0.1147$, $n=435$, $p<0.05$) and the females ($r=0.1164$, $n=534$, $p<0.01$). Such correlations are significant in about one third of the age subgroups in Whites, but follow no ordered pattern. Total protein and uric acid do not correlate significantly in any Negro group studied. These correlations are tabulated in a later section (Table 21) under uric acid.

Total protein and hemoglobin correlate highly in a positive sense in Whites of combined sexes and females, but do not correlate significantly in White males ($r=0.0611$, $n=525$), Negroes ($r=0.1236$, $n=54$), Negro males ($r=0.2400$, $n=18$), or Negro females ($r=0.2573$, $n=36$). Further consideration of Whites and White females by age is shown below in Table 17.

This table shows that the highly significant correlations for total protein and hemoglobin are concentrated between ages 25 and 44 years in both the Whites of combined sexes and females alone. Only the combined sex group aged 75 and over is also significant, and furthermore, White males show no significant correlation for total protein and hemoglobin for similar ages of 25-44 and 75 and over.

Negroes of either sex do not show a significant correlation between total protein and plasma, but do show positive correlations significant at the 1.0-0.1% level between total protein and the four globulin fractions when studied by either sex or age. The only exception to this is between total protein and alpha-1 globulin for which the correlation is not significant in two instances, a negative value in Negro males ([illegible], n=13) and a positive [illegible] and 15-24 [illegible], n=31).

Correlations between total protein and uric acid are positive and significant in either the combined sexes [illegible], p< 0.001), the males (r=0.1147, n=425, p< 0.05) and the females (r=0.1164, n=524, p<0.01). Such correlations are significant in about one third of the age subgroups in Whites, but follow no ordered pattern. Total protein and uric acid in most particular physiologically may begin early [illegible]. These correlations are tabulated in a later section (Table 21) under uric acid.

Total protein and hemoglobin correlate highly in positive sense in whites of combined sexes and females, but do not correlate significantly in white males [illegible], p<0.1), Negroes [illegible], p<0.1), Negro males [illegible], n=13), or Negro females (r=0.2316, p<0.1). Further quantitation of Negro and White females by age is shown below in Table 17.

While table shows that the highly significant correlations for total protein and hemoglobin are maintained between ages 25 and 44 years in both the White and Negro [illegible] females alone. Only the combined sex group aged 15 and over is highly significant, and females alone or significant correlation. The total negative but similar ages of 25-44 and 75 and over.

Table 17

Correlation Coefficients (r) for
Total Protein and Hemoglobin in Whites

Age	Sexes Combined			White Females Only		
	n	r	p	n	r	p
All ages	915	0.1285	0.01	507	0.1214	0.01
21-24	54	-0.0117	NS	32	0.0640	NS
25-34	142	0.2780	0.01	77	0.3871	0.001
35-44	189	0.1937	0.01	107	0.2599	0.01
45-54	247	-0.0008	NS	133	-0.0183	NS
55-64	156	0.1271	NS	91	-0.0039	NS
65-74	102	0.1485	NS	54	0.2283	NS
75 & over	24	0.4063	0.05	13	0.3201	NS

Albumin. In addition to the significant direct correlation between albumin and total protein, albumin consistently correlates inversely with each of the four globulin fractions. These correlations differ little one fraction to the next for a given group, and the pattern remains consistent in each age subgroup. The correlations listed in Table 18 for the large White groups are each significant at the p 0.001 level. The correlations in Negro groups show similar correlation coefficients, but in view of the small sample sizes, the level of significance of these correlations is lower and more variable.

Table IV

Correlation Coefficients (r_p) for
Total Protein and Hemoglobin in Whites

n	r	p	n	t	P
36	-0.0317	NS	32	0.0800	NS
142	0.1760	0.01	77	0.3071	0.001
140	0.1951	0.01	107	0.2309	0.01
247	-0.0008	NS	135	-0.0183	NS
156	0.1211	NS	91	-0.0039	NS
102	0.1685	NS	54	0.2283	NS

Table 18

Correlation Coefficients(r) Between Albumin and the Globulin Fractions

Group	n	Alpha-1	Alpha-2	Beta	Gamma
*All Whites	973	-0.2731	-0.3652	-0.3338	-0.3435
*White Males	438	-0.2968	-0.3640	-0.3637	-0.4083
*White Females	535	-0.2416	-0.3373	-0.3050	-0.2824
All Negroes	56	-0.2580 $p < 0.10$	-0.3398 $p < 0.05$	-0.5024 $p < 0.001$	-0.3456 $p < 0.01$
Negro Males	18	-0.0027 p=NS	-0.1146 p=NS	-0.4867 $p < 0.05$	-0.4071 $p < 0.10$
Negro Females	38	-0.3133 $p < 0.10$	-0.3914 $p < 0.05$	-0.4948 $p < 0.01$	-0.3143 $p < 0.10$

*Each correlation significant $p < 0.001$

Correlation coefficients between albumin and uric acid are positive and significant in the total White group ($r=0.1962$, $n=969$, $p < 0.01$) and the White females ($r=0.0893$, $n=534$, $p < 0.05$), but not in White males or in Negroes of both or either sex. When considered by age subgroups, albumin and uric acid correlations continue at the 1% level of significance for Whites of combined sexes in all but the 75 and over year olds. However, in White males no age subgroup showed a significant correlation and only the 25-34 year old White females correlated significantly ($r=0.2838$, $n=85$, $p < 0.01$). It can also be noted that in the Whites of

Table 12

Intercorrelation Coefficients(*) Between
Albumin and the Globulin Fractions

Group	N	Alpha-1	Alpha-2	Beta	Gamma
All White	171	-0.1773	-0.4386	-0.1146	-0.3459
White Males	101	-0.1358	-0.3843	-0.2017	-0.4041
White Females	70	-0.2311	-0.5111	-0.0606	-0.2811
All Negroes	76	-0.2506	-0.3706	-0.3024	-0.3436
		$p < 0.10$	$p < 0.05$	$p < 0.05$	$p < 0.01$
Negro White	42	-0.0021	-0.1111	-0.0021	-0.2857
					$p < 0.10$
Negro Females	30	-0.3133	-0.3800	-0.4000	-0.3143
		$p < 0.10$	$p < 0.05$	$p < 0.01$	$p < 0.10$

*Each correlation
significant $p < 0.001$

Discussion

Comparing correlations between albumin and beta and beta globulin in the Negro females (r=0.4000, $p < 0.01$), but not in White males or in Negroes of both or either sex. When considered by age subgroups, albumin and beta globulin correlations continue at the .05 level of significance for Negroes of combined sexes in all but the 21 and over year interval. However, in White males by age subgroup albumin and beta globulin correlations are significant only for the 21-30 year old White females correlated significantly ($r = -0.5111$, $p < 0.01$). It was also noted that in the Negro of

combined sexes aged 25-34, the level of significance attained is 0.1% rather than 1%. In Negroes, albumin and uric acid correlated at the 5% level only when divided by age, r being positive under 35 and negative in those 35 and older. All these correlations, including the age subgroups for the combined sexes in Whites, are listed in a later table (Table 21) under uric acid correlations.

Albumin and hemoglobin do not correlate significantly in any of the Negro age or sex groups, but do correlate positively and significantly in combined sexes in Whites ($r=0.2489$, $n=915$, $p < 0.001$), White males ($r=0.1150$, $n=408$, $p < 0.05$), and in White females ($r=0.1498$, $n=507$, $p < 0.01$). When Whites are divided by age (see Table 19), in the combined sexes significance is maintained in all but the 75 and over category, in White females in all but those aged 45-54 (in 21-24 year olds, only the 10% level is attained), but in White males no significant correlation is found in any age subgroup. It can be noted that in each of the three White groups, some of the lowest correlations between albumin and hemoglobin are found in ages 45-54.

Gobulin fractions. The significant direct correlation between the four globulin fractions and total protein and the inverse correlation with albumin have already been described above. The globulin fractions themselves correlate highly one with another, and the ranges of values of r for the six large groups of sexes separate or combined are 0.4022 to 0.6505 in Whites and -0.2681 to 0.8423 in Negroes. Excluding the negative values which are confined to each alpha-1 correlation in Negro males (none of which reach significant levels and are possible due to sampling), the Negro range becomes 0.3478 to 0.8423. In general, even with age subgroups considered separately, each globulin correlates more

rather than IL. In Negroes, albumin and uric acid correlated at the
IL level only when divided by age, P being positive under 35 and negative
in those 35 and older. All these correlations, including the age sub-
groups for the remaining sexes in Whites, are listed in a later table
(Table 21) under uric acid correlations.

Albumin and hemoglobin do not correlate significantly in any of the
Negro age or sex groups, but do correlate positively and significantly
in combined sexes in Whites ($r=0.2469$, $n=715$, $p<0.001$), White males
[illegible], and in White females ($r=0.1847$, $p<$
$p<0.01$). When Whites are divided by age (see Table 19), in the corre-
[illegible]
in White females in all but those aged 45-54 (in 21-24 year olds, only
[illegible]
is found in any age subgroup. It can be noted that in each of the three
[illegible]
ginals are found in ages 45-54.

Globulin Fractions: The significant direct correlations between the
globulin fractions and total protein and the inverse correlation with
[illegible]
saliva correlate highly one with another, and the ranges of values of
[illegible]
to 0.6505 in Whites and -0.2651 to 0.3823 in Negroes. Including the
negative values which are confined to each alpha-1 correlation in Negro
[illegible]
[illegible]
reading), the beta range becomes $r=.3819$ to 0.8427. In general, each

Correlation Coefficients for Albumin and Hemoglobin in Whites by Age and Sex

		① Alb and Hgb r	② n	③ Signif. Level	④	⑤
1340	ITES (BOTH SEXES)	0.2489	915	0.1%		
	AGED					
	1-24 YEARS	0.4823	54	0.1%		
	5-34	0.3861	142	0.1%		
	-44	0.3359	189	0.1%		
	15-54	0.1247	247	5%		
	5-64	0.2738	156	0.1%		
	5-74	0.2469	102	5%		
	75-89	0.2308	24	NS		
60	WHITE MALES	0.1150	408	5%		
	AGED					
	21-24 YEARS	0.3455	22	NS		
	25-34	0.1042	65	NS		
	35-44	0.1429	82	NS		
	45-54	-0.0233	114	NS		
	55-64	0.1901	65	NS		
	65-74	-0.0035	48	NS		
	75-88	-0.0488	11	NS		
73	WHITE FEMALES	0.1498	507	1%		
	AGED					
	-24 YEARS	0.3146	32	10%		
	-34	0.3091	77	1%		
	-44	0.2473	107	~1%		
	-54	0.0111	133	NS		
	-64	0.2455	91	5%		
	-74	0.2819	54	5%		
	-89	0.8232	13	0.1%		

highly with those of more similar electrophoretic mobilities. There is some variation in this pattern found at times with an increased correlation between alpha-1 and gamma and a decreased correlation found at other times between alpha-2 and beta as compared with other globulin correlations for a given group studied. As an example of this overall pattern, the various correlations among globulin fractions for the total White group is given below.

Table 20

Correlation among Globulin Fractions in 973 Whites

	alpha-1	alpha-2	beta	gamma
Alpha-1	1.0000			
Alpha-2	0.5250	1.0000		
Beta	0.4567	0.5078	1.0000	
Gamma	0.4316	0.4590	0.6023	1.0000

When the data are considered on a race or sex specific basis no significant correlation for any globulin fraction and uric acid is observed. Similarly, when considering correlations for globulins and hemoglobin by race and sex, the only significant correlations are negative and are found between alpha-2 and hemoglobin in Whites of combined sexes ($r = -0.1073$, $n=914$, $p < 0.01$) and White males ($r = -0.0984$, $n=408$, $p < 0.05$). If these significant correlations between alpha-2 globulin and hemoglobin are further examined by age, the combined sexes show a significance at the 1% level in ages 21-24 ($r = -0.3600$, $n=54$) and the White males show 5% significance levels in three age subgroups, 21-24 ($r = -0.4487$, $n=22$), 35-44 ($r = -0.2228$, $n=82$) and in 55-64 ($r=0.2677$, $n=65$). In this last group of White males, note that the relationship is a direct rather than an inverse one.

slight mid class of more acidic electrochemistry modifiers. There is some variation in this pattern found at times with an increased correlation between alpha-1 and gamma and a decreased correlation found in other times between alpha-2 and beta as compared with other classic interactions for a given group studied. In an example of this partial pattern, the matrix correlations among phenolic fractions for the test water group is given below.

Table 2C

Correlations among Phenolic Fractions in PCP Groups

	alpha-1	alpha-2	beta	gamma
Alpha-1	1.0000			
Alpha-2	0.2250	1.0000		
Beta	0.4567	0.5078	1.0000	
Gamma	0.4316	0.4350	0.6023	1.0000

When the data are considered on a tree or bar specific basis no significant correlations for any phenolic fraction and site exist in the matrix. Similarly, when considering correlations for phenolics and groupings by zone and age, the only significant correlations are negative and are found between alpha-1 and chemistry in Unit 2 of combined zones ($r = -0.1217$, $n = 114$, $p > 0.05$) and Unit 1 ($r = -0.0804$, $n = 114$, $p > 0.05$). If these significant correlations between alpha-1 chemistry and homogeneous Zone Units measured by age, the combined zones show a significance at the 1% level in Unit 1 ($r = -0.1800$, $n = 81$) and the Units matrix show 1% significance levels in three age categories, 11-16 ($r = -0.4827$, $n = 17$), 31-46 ($r = -0.1318$, $n = 32$) and in 16-30 ($r = -0.1917$, $n = 65$). In this last group of Units mains, note that the relationship is a direct rather than an inverse one.

Uric Acid. Table 21 lists by race, sex and age the correlations of serum uric acid with each serum protein studied and also hemoglobin. In Whites, total protein correlates positively and significantly with uric acid when studied by sex, but when the factor of age is considered, the significant correlation is absent in many subgroups. In the combined sexes group in Whites, albumin correlates significantly and positively with uric acid in every age subgroup but those over 74, while in the separate sexes there is but a single female subgroup (aged 25-34) which is significant. Uric acid and hemoglobin correlate directly with high significance in the combined sexes in Whites both for all ages as well as for separate ages. This significant correlation is present for each sex if all ages are considered together, but if ages are separated the significance is irregular.

Hemoglobin. Correlations between hemoglobin and the serum protein and serum uric acid levels have been discussed under those sections. Most notable are the correlations between hemoglobin and total protein (p.72) in White females and those of combined sexes, between hemoglobin and albumin (p. 75) in each White group of combined ages, but only in the combined sexes and the females when age subgroups are considered separately. Hemoglobin and uric acid (above) correlate significantly in each combined age group of Whites, but in age subgroups only the combined sexes correlate consistently, while the separate male and female age subgroups show a varied pattern of significance.

Correlation Coefficients (r) Between Uric Acid and Serum Proteins and Hemoglobin: Race, Sex and Age Groupings.

Between Uric acid and:	Total r	Protein * n	Signif level r	Albumin r	n	Signif level r	Alpha 1 r	Globulin n	Signif. level r	Alpha 2 r	Globulin n	Signif. level r	Beta r	Globulin n	Signif. level r	Gamma r	Globulin n	Signif. level r	Hemoglobin r	n	Signif. level r
1340 WHITES (Both sexes)	0.1571	969	<<1%	0.1962	969	<<1%	-0.0186	967		-0.0508	967		0.0299	967		-0.0031	967		0.4075	1173	<<1%
72 aged 21-24 years	0.0341	55		0.3676	55	1%	-0.0762	55		-0.2072	65		-0.1696	65		-0.2195	55		0.5166	65	<<1%
214 " 25-34 "	0.1373	159		0.3436	159	0.1%	-0.0635	159		-0.2217	159	<1%	-0.0355	157		-0.0675	159		0.5188	180	<<1%
281 " 35-44 "	0.1677	196	5%	0.2535	196	1%	-0.0487	195		-0.0422	195		0.0445	195		-0.0422	195		0.5177	249	<<1%
361 " 45-54 "	0.2204	258	1%	0.2146	258	1%	0.0439	258		0.0646	258		0.0819	258		-0.0510	258		0.3892	324	<<
228 " 55-64 "	0.1270	170		0.2380	170	1%	-0.1475	169	~5%	-0.1679	169	5%	-0.0206	169		0.0487	169		0.3084	200	<<
147 " 65-74 "	0.1340	105		0.3026	105	1%	-0.0149	105		-0.0914	105		-0.0747	105		-0.0851	105		0.1683	122	
36 " Over 74 "	0.6457	25	0.1%	0.1125	25		0.0355	25		0.2539	25		0.0662	25		0.4166	25	<5%	0.4119	32	
605 White Males	0.1147	435	5%	0.0633	435		0.0220	435		-0.0126	435		0.0123	435		0.0588	435		0.1733	531	1%
28 aged 21-24 yrs.	-0.0022	22		0.2623	22		-0.0157	22		-0.0626	22		-0.1183	22		-0.1204	22		0.1553	28	
97 " 25-34 "	-0.0435	74		0.1330	74		-0.1072	74		-0.3131	74	1%	-0.0332	74		-0.0581	74		0.2533	81	
123 " 35-44 "	0.0904	85		0.1309	85		-0.0095	85		0.0244	85		0.0334	85		-0.0235	85		0.2225	112	
166 " 45-54 "	0.2498	119	1%	-0.0163	119		0.1660	119		0.1395	119		0.1176	119		0.1183	119		0.1952	150	
102 " 55-64 "	0.1079	75		0.1768	75		-0.0517	75		-0.1426	75		-0.0576	75		0.0290	75		0.1302	87	
68 " 65-74 "	0.0708	47		0.1844	47		-0.0292	47		-0.0120	47		0.0001	47		-0.0841	47		0.1208	55	
20 " over 74 "	0.6352	12	5%	0.5361	12		-0.2543	12		-0.0406	12		0.0385	12		0.4502	12		0.3220	17	
735 White Females	0.1164	534	1%	0.0893	534	5%	0.0066	532		0.0397	532		0.0329	532		0.0189	532		0.1518	642	<1%
44 aged 21-24 years	0.0236	33		0.1322	33		-0.1466	33		-0.0840	33		-0.1198	33		-0.1331	33		0.4693	37	1%
117 " 25-34 "	0.2155	85	5%	0.2228	85	1%	0.0974	85		-0.0761	85		0.0308	85		0.0189	85		0.0410	99	
158 " 35-44 "	0.1124	111		0.0579	111		-0.0251	110		0.0322	110		0.0335	110		0.1036	110		0.0750	137	
195 " 45-54 "	0.1365	139		0.1619	139		0.0174	139		0.1318	139		0.0226	139		-0.0714	139		0.1854	174	5%
126 " 55-64 "	0.1769	95		0.1623	95		-0.0448	94		-0.0551	94		0.0095	94		0.1084	94		0.1211	113	
79 " 65-74 "	0.0279	58		0.2314	58		-0.1485	58		-0.1424	58		-0.0444	58		-0.1247	58		-0.1340	67	
16 " over 74 "	0.5715	13	5%	0.0131	13		0.2620	13		0.4539	13		0.3095	13		0.1238	13		0.1234	15	
85 Negroes (Both sexes)	-0.0376	57		-0.0013	57		-0.0498	56		-0.1378			-0.0825	56		0.0486	56		0.4142	74	<1%
39 aged 21-34 years	-0.0471	23		0.4622	23	5%	-0.0664	23		-0.3784			-0.1878	23		-0.2446	23		0.5113	34	<1%
46 " 35-76 "	-0.0508	34		-0.3798	34	5%	0.0921	33		0.0445			0.1146	33		0.2846	33		0.2464	40	
31 Negro Males	-0.0343	19		-0.4066	19		-0.0183	18		0.1079			0.1950	18		0.3379	18		0.1294	25	
54 Negro Females	0.0454	38		0.0437	38		-0.0317	38		-0.0537			0.0035	32		0.6710	38		0.3393	49	5%

* n = sample size

Multiple Regression Analysis

Two sets of multiple regression analyses are presented in the following two tables. Each set considers for 419 White males and 536 White females various combinations of the following parameters: age, hemoglobin, albumin, the four globulin fractions (alpha-1, alpha-2, beta and gamma) and uric acid.

The first set places uric acid in the role of dependent variable. In males, hemoglobin, age and albumin are in that order the best predictors of uric acid, with the four globulin fractions contributing little additional. In females, uric acid can be best predicted by age, albumin and hemoglobin in that order, with little contribution from the globulin fractions. In addition, using age as a sole independent variable lowers the multiple correlation coefficient R little, and produces the largest F-ratio of the set.

In the second set of analyses, hemoglobin is the dependent variable. In order of predictive value, in males are uric acid, albumin and age (the latter having a negative regression coefficient), with the globulins of no significance. In females, age, albumin and also uric acid are good predictors of hemoglobin, accounting for approximately 9% of its variability. Age and albumin paired as independent variables allow estimation of hemoglobin nearly as well as the combination of all seven variables, and age, albumin and uric acid each taken alone are significant parameters. Again, the globulin fractions contribute no significance over that of the three factors indicated.

Two sets of multiple regression analyses are presented in the following two tables. Each set considers for 419 White males and 336 White females the five plasmatic direction lipids-I, albumin, hemo and uric acid.

The first set places uric acid in the role of dependent variable. In males, hemoglobin, age and albumin are in that order the best predictors of uric acid, with the four globulin fractions contributing little additional. In females, uric acid can be best predicted by age, albumin and hemoglobin in that order, with little contribution from the globulin fractions. In addition, using age as a male independent variable lowers the multiple correlation coefficient a little, and produces the largest F-ratio of the set.

In order of predictive value, in males are uric acid, albumin and age of no significance. In females, age, albumin and also uric acid are good predictors of hemoglobin, somewhat low consistently if of its sensitivity. The red albumin paired as independent variable also contribution of hemoglobin nearly as well as the combination of all seven variables, and are. Albumin and uric acid were [illegible] parameters. Under the plasmatic fractions contribute no significance over that of the three factors indicated.

Correlation Coefficients (r) Between Uric Acid and Serum Proteins and Hemoglobin: Race, Sex and Age Groupings.

Between Uric acid and:	Total r	Protein * n	Signif level r	Albumin r	n	Signif level r	Alpha 1 r	Globulin n	Signif level r	Alpha 2 r	Globulin n	Signif. level r	Beta r	Globulin n	Signif. level r	Gamma r	Globulin n	Signif. level r	Hemoglobin r	n	Signif. level r
1340 WHITES (Both sexes)	0.1571	969	<<1%	0.1962	969	<<1%	-0.0186	967		-0.0508	967		0.0299	967		-0.0031	967		0.4075	1173	<<1%
72 aged 21-24 years	0.0341	55		0.3676	55	1%	-0.0762	55		-0.2072	55		-0.1696	55		-0.2195	55		0.5166	65	<<1%
214 " 25-34 "	0.1373	159		0.3436	159	0.1%	-0.0635	159		-0.2217	159	<1%	-0.0355	159		-0.0675	159		0.5188	180	<<1%
221 " 35-44 "	0.1677	196	5%	0.2535	196	1%	-0.0487	195		-0.0422	195		0.0445	195		-0.0422	195		0.5177	249	<<1%
301 " 45-54 "	0.2204	258	1%	0.2146	258	1%	0.0439	258		0.0646	258		0.0659	258		-0.0510	258		0.3812	324	<<1%
238 " 55-64 "	0.1270	170		0.2380	170	1%	-0.1475	169	~5%	-0.1679	169	5%	-0.0206	169		0.0487	169		0.3834	200	<<1%
147 " 65-74 "	0.1340	105		0.3026	105	1%	-0.0149	105		-0.0914	105		-0.[illegible]47	105		-0.0851	105		0.1663	122	
36 " Over 74 "	0.6457	25	0.1%	0.1125	25		0.0355	25		0.2589	25		0.[illegible]62	25		0.4166	25	<5%	0.4119	32	
605 White Males	0.1147	435	5%	0.0638	435		0.0220	435		-0.0126	435		0.0[illegible]23	435		0.0588	435		0.1733	531	1%
128 aged 21-24 yrs.	-0.0022	22		0.2623	22		-0.0157	22		-0.0626	22		-0.[illegible]883	22		-0.1204	22		0.1563	28	
197 " 25-34 "	-0.0435	74		0.1330	74		-0.1072	74		-0.3131	74	1%	-0.[illegible]82	74		-0.0581	74		0.2533	81	
123 " 35-44 "	0.0904	85		0.1309	85		-0.0095	85		0.0244	85		0.[illegible]34	85		-0.0235	85		0.2225	112	
166 " 45-54 "	0.2498	119	1%	-0.0163	119		0.1660	119		0.1395	119		0.[illegible]76	119		0.1183	119		0.1952	150	
102 " 55-64 "	0.1079	75		0.1768	75		-0.0517	75		-0.1426	75		-0.[illegible]76	75		0.0290	75		0.1362	87	
68 " 65-74 "	0.0708	47		0.1844	47		-0.0292	47		-0.0120	47		0.[illegible]01	47		-0.0841	47		0.1208	55	
20 " over 74 "	0.6352	12	5%	0.5361	12		-0.2643	12		-0.0406	12		0.[illegible]85	12		0.4502	12		0.3220	17	
735 White Females	0.1164	534	1%	0.0893	534	5%	0.0066	532		0.0397	532		0.[illegible]29	532		0.0189	532		0.1518	642	<1%
44 aged 21-24 years	0.0236	33		0.1822	33		-0.1466	33		-0.0840	33		-0.[illegible]98	33		-0.1331	33		0.4693	37	1%
117 " 25-34 "	0.2155	85	5%	0.2838	85	1%	0.0974	85		-0.0761	85		0.[illegible]08	85		0.0189	85		0.0410	99	
158 " 35-44 "	0.1124	111		0.0579	111		-0.0251	110		0.0322	110		0.[illegible]85	110		0.1036	110		0.0750	137	
195 " 45-54 "	0.1365	139		0.1619	139		0.0174	139		0.1318	139		0.[illegible]026	139		-0.0714	139		0.1854	174	5%
126 " 55-64 "	0.1769	95		0.1623	95		-0.0448	94		-0.0551	94		0.[illegible]095	94		0.1084	94		0.1211	113	
79 " 65-74 "	0.0279	58		0.2314	58		-0.1485	58		-0.1424	58		-0.[illegible]444	58		-0.1247	58		-0.1340	67	
16 " over 74 "	0.5715	13	5%	0.0131	13		0.2620	13		0.4539	13		0.3095	13		0.1238	13		0.1234	15	
85 Negroes (Both sexes)	-0.0376	57		-0.0013	57		-0.0498	56		-0.1378			-0.0825	56		0.0484	56		0.4142	74	<1%
39 aged 21-34 years	-0.0471	23		0.4622	23	5%	-0.0664	23		-0.3784			-0.1878	23		-0.2446	23		0.5113	34	<1%
46 " 35-76 "	-0.0508	34		-0.3798	34	5%	0.0921	33		0.0445			0.1146	33		0.2846	33		0.2464	40	
31 Negro Males	-0.0343	19		-0.4066	19		-0.0183	18		0.1079			0.1950	18		0.3379	18		0.1294	25	
54 Negro Females	0.0454	38		0.0437	38		-0.0317	38		-0.0537			0.0035	38		0.0710	38		0.3393	49	5%

* n = sample size

Multiple Regression: Uric Acid as Dependent Variable in Whites by Sex

	b	t	b	t	b	t	b	t	b	t
419 White ♂ 1 = age	0·01144	2·8881	0·01148	2·9741	0·0147	2·9660			0·0136	2·7066	0·01101	2·1408
2 = hemoglobin	0·1688	3·2853	0·1748	3·4014	0·1756	3·4231	0·1648	3·1827				
3 = albumin	0·2955	2·0377	0·20219	1·4579	0·2178	1·6422	0·0761	0·5692	0·2678	2·0062		
4 = α_1 globulin	0·1687	0·2649										
5 = α_2 globulin	−0·7111	−1·4792	−0·1408	−0·3574			−0·0974	−0·2450				
6 = β globulin	0·5636	1·3789										
7 = δ globulin	0·2397	0·8958										
degrees of freedom	411		414		415		415		416		417	
R = mult. correl. coefficient	0·245437		0·218048		0·217373		0·164911		0·142651		0·104266	
F ratio =	3·763616		5·166525		6·860548		3·867218		4·320568		4·583179	
Intercept =	1·222584		1·965878		1·812240		3·461392		4·30404		5·872235	
536 White ♀ 1 = age	0·0235	6·4101	0·0236	6·4439	0·0235	6·4108			0·0255	7·1564	0·0229	6·5403
2 = hemoglobin	0·0816	2·1177	0·0834	2·1711	0·0866	2·2543	0·1443	3·7341				
3 = albumin	0·3699	3·2781	0·3350	3·0552	0·2720	2·6404	0·1522	1·3855	0·3237	3·2113		
4 = α_1 globulin	−0·2307	−0·4551										
5 = α_2 globulin	0·3294	0·9240	0·5000	1·6569			0·4728	1·5103				
6 = β globulin	0·3374	1·1682										
7 = δ globulin	0·0855	0·4924										
degrees of freedom	582		531		532		532		533		534	
R = mult. correl. coeff.	0·330396		0·323971		0·316749		0·186991		0·302879		0·272328	
F ratio =	9·242888		15·56690		19·77590		6·425233		26·91673		42·77519	
Intercept	0·2733799		0·5728185		1·106350		1·764779		1·931320		3·656373	

MULTIPLE REGRESSION: HEMOGLOBIN AS DEPENDENT VARIABLE IN WHITES BY SEX

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)
Independent Variable	b	t	b	t	b	t	b	t	b	t	b	t	b	t	b	t	b	t	b	t	
419 White ♂ 1 = age	-0.0086	-1.8215	-0.0085	-1.7987			-0.0087	-1.8350			-0.0119	-2.6811	-0.0065	-1.3792					-0.0102	-2.2835	
2 = uric acid	0.1516	3.2853	0.1555	3.4014	0.1446	3.1827	0.1564	3.4231	0.1454	3.2005	0.1651	3.6188					0.1523	3.3329			
3 = albumin	0.2511	1.8255	0.2117	1.6138	0.2876	2.3097	0.2430	0.1944	0.3236	2.7579			0.2869	2.2614	0.3445	2.9085					
4 = α_1 globulin	0.2148	0.3561																			
5 = α_2 globulin	-0.4767	-1.0450	-0.2937	-0.7906	-0.3224	-0.8661															
6 = β globulin	-0.2228	-0.5742																			
7 = δ globulin	0.3109	1.2273																			
degrees of freedom	411		414		415		415		416		416		416		417		417		417		
R = mult. correl. coeff:	0.237687		0.229275		0.212517		0.226133		0.208416		0.206139		0.156027		0.141007		0.161083		0.111133		
F ratio =	3.515704		5.742647		6.543096		7.455089		9.445211		9.230883		5.199959		8.459419		11.10849		5.214588		
Intercept =	13.54346		13.81928		13.10367		13.51808		12.75771		14.89006		14.19120		13.57087		14.41211		15.85949		
536 White ♀ 1 = age	0.0207	4.9516	0.0273	4.9591			0.0206	4.9363			0.0153	3.7152	0.0234	5.8444					0.0186	4.6496	
2 = uric acid	0.1032	2.1177	0.1055	2.1711	0.1770	3.7341	0.1092	2.2543	0.1800	3.8077	0.1422	2.8995					0.1918	4.0170			
3 = albumin	0.6357	5.0814	0.6047	4.9730	0.4545	3.7768	0.5617	4.9343	0.4173	3.7126			0.5971	5.2752	0.4459	3.9266					
4 = α_1 globulin	0.7886	1.3861																			
5 = α_2 globulin	-0.0004	-0.0010	0.3419	1.0056	0.3011	0.8671															
6 = β globulin	0.2464	0.7583																			
7 = δ globulin	-0.0609	-0.3120																			
degrees of freedom	528		531		532		532		533		533		533		534		534		534		
R = mult. correl. coeffs	0.319399		0.311384		0.234810		0.308610		0.231949		0.232021		0.294274		0.167518		0.171266		0.197254		
F ratio =	8.569084		14.25347		10.34795		18.66712		15.15306		15.16296		25.26620		15.41784		16.13668		21.61870		
Intercept =	3.606807		8.932585		10.32436		9.311164		10.65202		12.18399		9.522095		11.36067		12.66318		12.70374		

VI. Summary, Discussion and Conclusions

The Survey

The sample for this study comes from the New Haven Arthritis Survey, and as far as is known comprises the largest single group other than hospital patient series in which serum proteins have been studied by electrophoresis of any type. There were sera from 1029 adult individuals (973 Whites, 56 Negroes); the only group which approaches this in size is that of 1005 sera studied in the Philippines by Samson et al (1965), but this series also contained cord blood samples from newborns, however, as well as the range of ages younger than those studied in the New Haven sample. There are few other surveys where the full range of adult ages is studied for serum proteins by paper electrophoresis, and only one other among Caucasians, namely that of Nilsson et al (1964) in 207 Swedes, of a general population living at home and not screened for either health or disease (See pages 8,9,14,15). Pollak (1961) used paper electrophoresis of serum proteins as the basis for his study of Caucasians and Negroes living in similar environments.

The New Haven sample is not complete, because only 61% of the total population was tested for blood, urine or radiographic findings. Of these only 71% had blood analyses complete for all factors studied here, while 88% had at least hemoglobin and 94% at least uric acid determinations.

Three serum protein studies use the Spinco method of paper electrophoresis, Pollak (1961) in 62 Caucasians and 62 Negroes, Samson (1965) in 1005 Filipinos, and Kirkeby (1966) in 170 Norwegians. The Spinco

method is also used in the present study, although some change was made in the strength of the constant current and in the substitution of lissamine green for bromphenolblue as a protein dye. Bovine serum albumin was used as recommended as the protein standard for the independent determination of total protein by the biuret method. Samson noted that Veratol-A was used in their study, but we found no difference for total protein when either standard was used. However, beeause of the choice of lissamine green as protein dye on the electrophoresis strips, it was also chosen to determine serum albumin independently (by the HABA dye method) on the basis of the bovine albumin standard as mentioned in an earlier section. Unfortunately, this standard does produce albumin curves which describe albumin levels significantly higher (0.5-1.5 g/100 ml) than those determined with other standards. Furthermore, the levels of individual globulins are thereby lower with this standard, since in this study total globulin is defined as the difference between total protein and albumin as determined by the methods described.

Although the mean values for serum proteins found in the present study cannot be used in direct comparison with those of other studies in view of the methodological bias described, nevertheless the method was consistent throughout the analyses so that trends and differences within the sample itself can be considered valid. Another point which should be noted is that serum protein levels are known to be greater in a person when he is ambulatory than when he is at bedrest. Lange (1946) found that total protein was increased 8% in a person when he was up than when at bedrest, and was increased an additional 6 - 12% after

brief, vigorous exercise. Among 20 students Aull and McCord (1957) found an average increase in total protein levels of 0.88 g /100 ml (11.6%) after 2 - 3 hours of laboratory class compared with serum total proteins drawn before arising from bed, and the increase was approximately proportional among the albumin, alpha, beta and gamma globulin fractions.

Serum Protein Mean Values. For convenient reference the mean serum protein values found in this study and those of the three studies mentioned using electrophoresis method differing only in albumin determination and choice of protein dye as described, figures 10-17 were prepared showing means by race and sex where available data k permitted. These include Whites and Negroes of each sex from this study, 170 Norwegians of both sexes (Kirkeby, 1966), 62 Caucasian adults and 62 Negro adults (50% of either sex in each group) (Pollak, 1961), and 165 Filipinos between 30-50 years old from the much larger Filipino sample (Samson, 1965). These eight sets of serum protein means ($\pm$ 2 S.D.) are plotted on scales which show in shaded area the range considered normal for serum proteins by paper electrophoresis in the Clinical Laboratory of the Yale-New Haven Hospital. These normal ranges represent the means $\pm$ 1 S.D. from unpublished data on the sera of 100 healthy members of the hospital staff. Total serum proteins in this group are 7.1 $\pm$ 0.3g/100 ml.

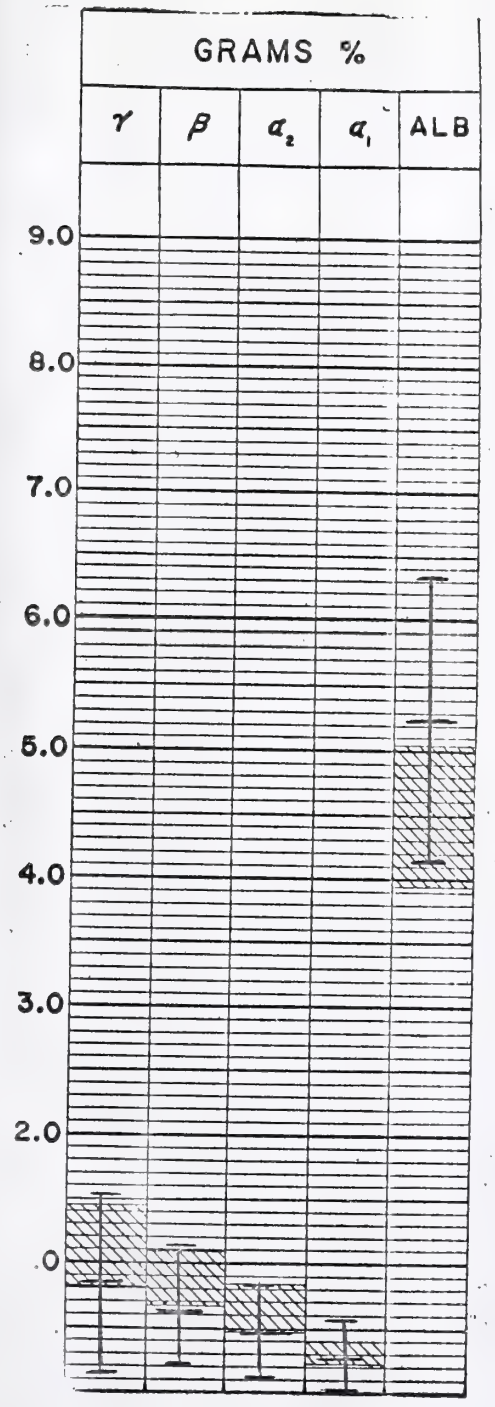


Fig. 10

8 White Males
resent Study)

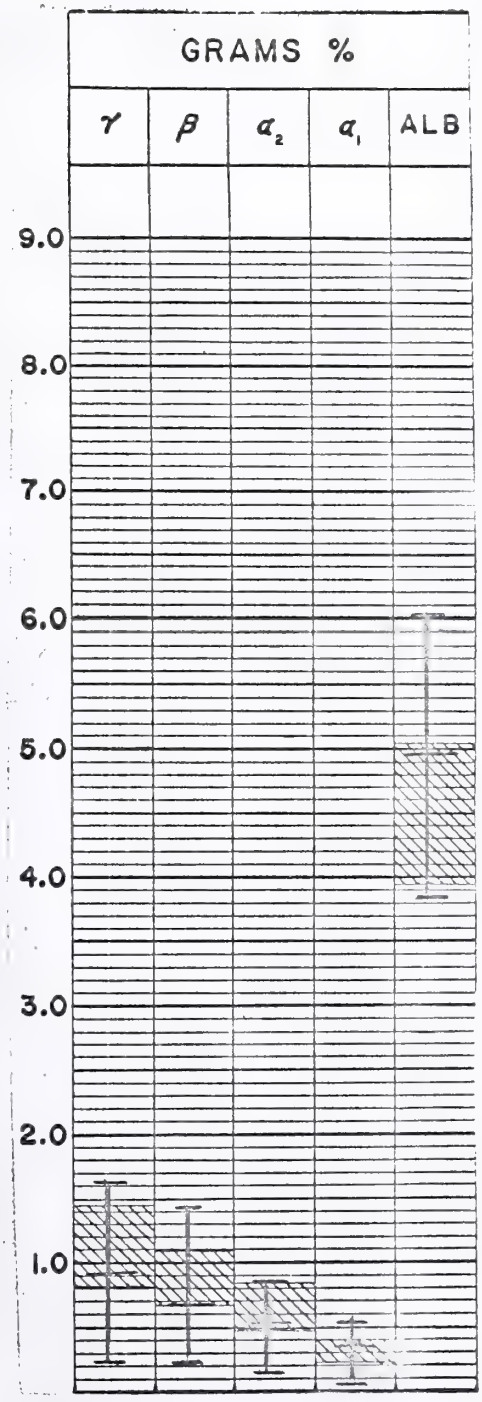


Fig 11.

535 White Females
(Present Study)

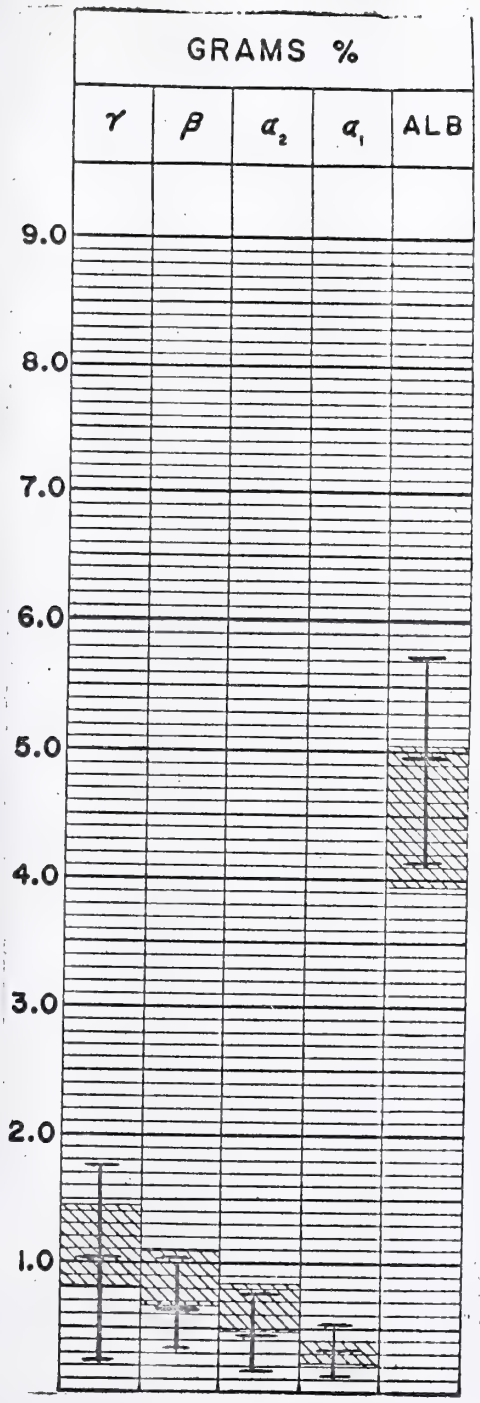


Fig. 12

18 Negro Males
(Present Study)

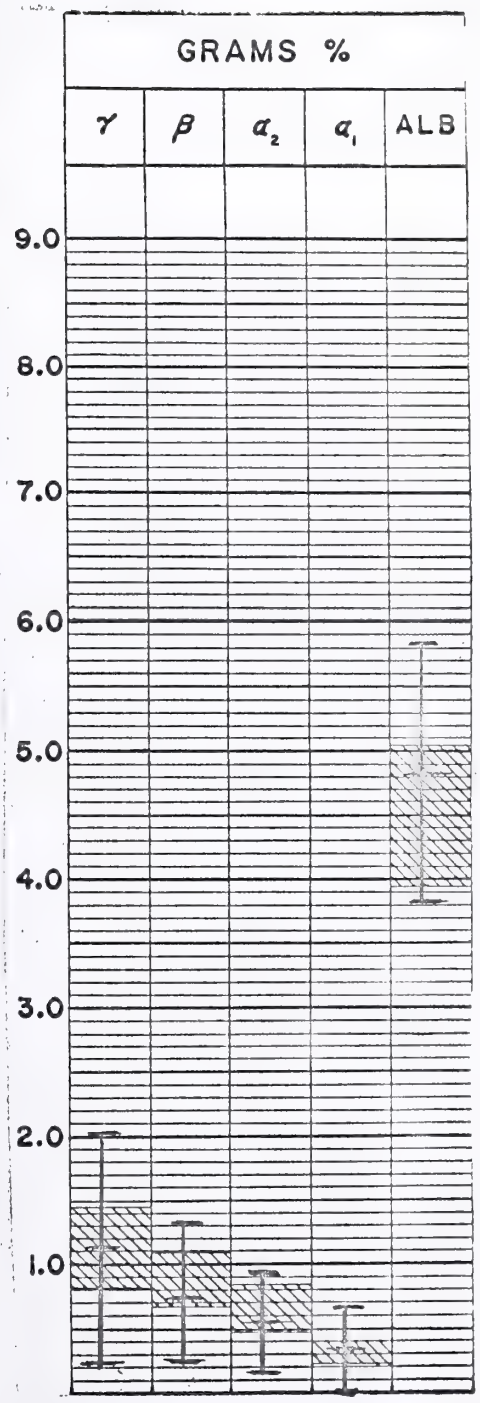


Fig. 13

38 Negro Females
(Present Study)

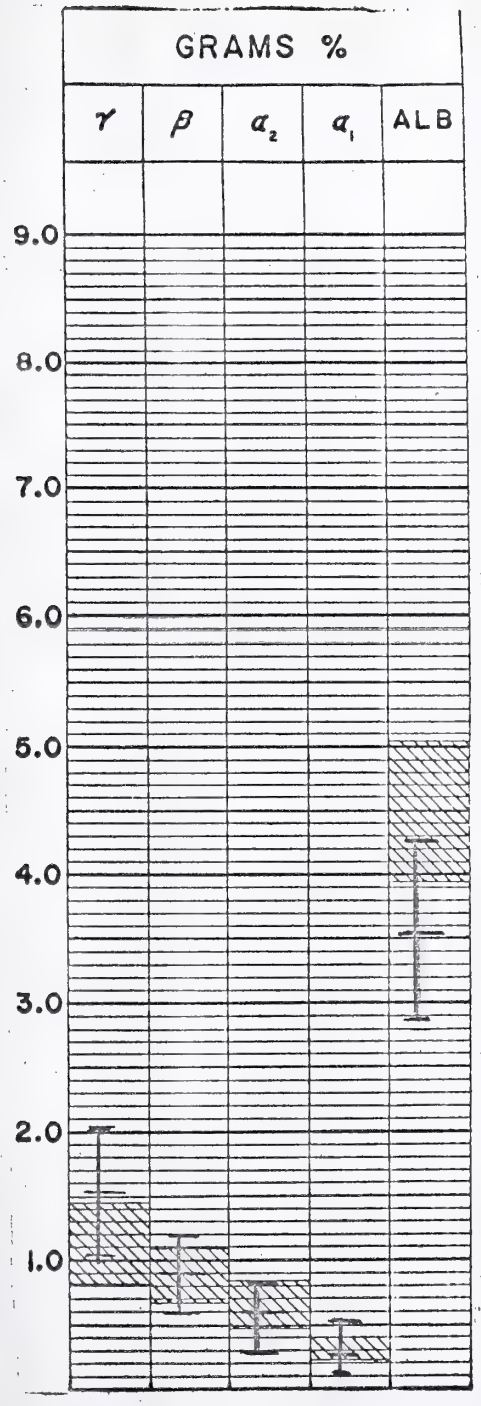


Fig. 14

170 Caucasians (Kirkeby, 1966)

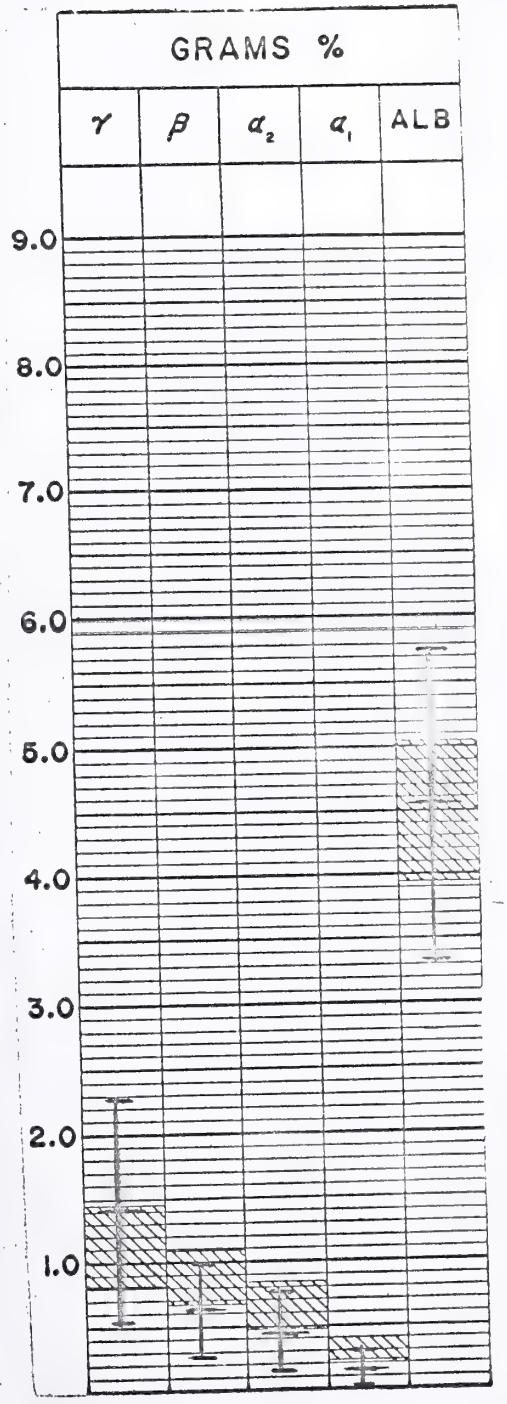


Fig. 15

165 Filipinos Aged 30-50 years (Samson,1965)

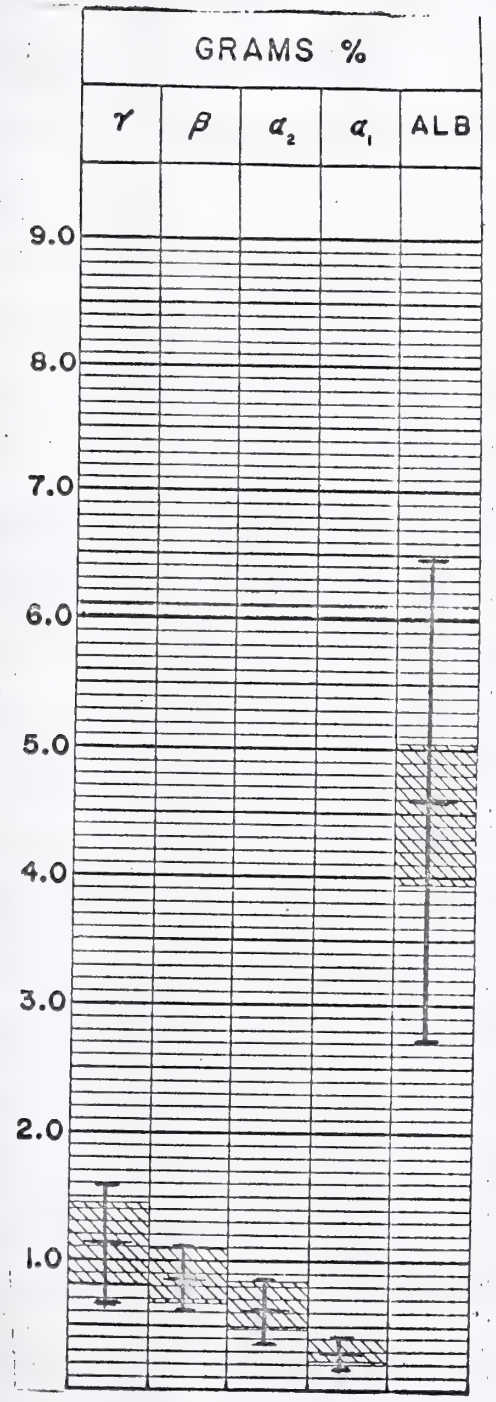


Fig. 16

62 Caucasians
(Pollak, 1961)

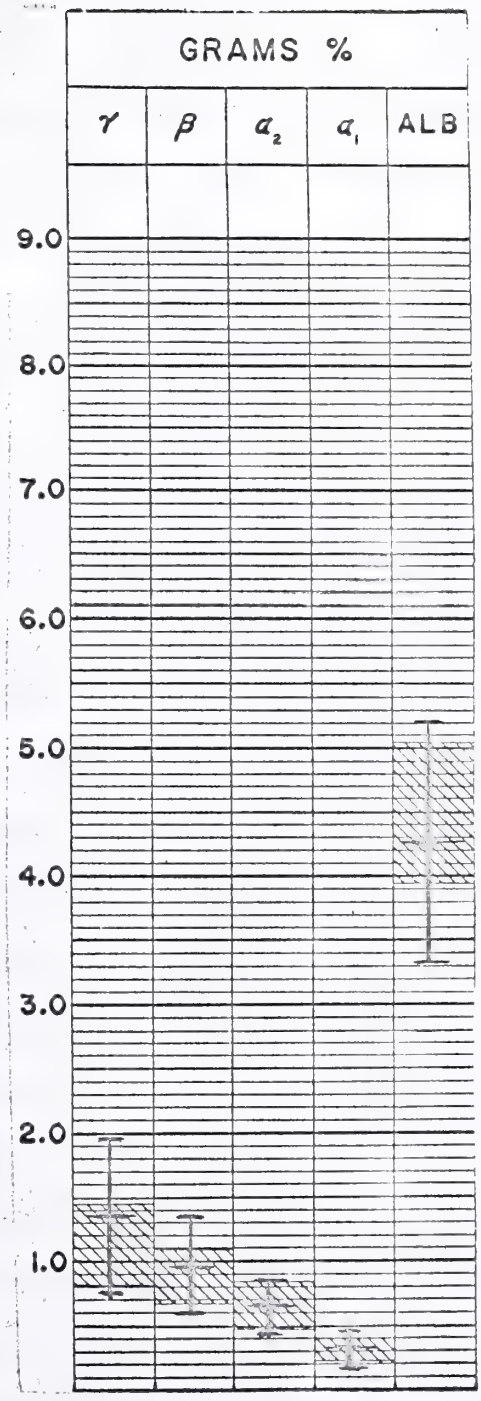


Fig. 17

62 American Negroes
(Pollak, 1961)

As can be clearly seen, albumins are distinctly higher and globulins lower in almost every case in the present findings, which can be expected in view of the methodological bias described above. It is clearly not indicated in this basic report that the methods of this study should be standardized against those of another laboratory or method and a correction factor be introduced into the data, nor does it seem likely that adding such a factor with its own inherent sources of error will contribute significantly to the value of the present data.

Serum Proteins and Race. The present study is in accord with the results of previous studies reviewed in finding a lower serum albumin in Negroes compared with Whites, and a higher total globulin, often due to differences in the gamma fraction. The findings are also in complete accord with those of Pollak (1961) who also carried out a paper electrophoresis study among Whites and Negroes in this country. Total proteins show little racial difference, although they are highest in Negro females and White males. Albumins are distinctly higher in Whites, and this difference is mostly accounted for by the high value in White males. Alpha-1 globulins are higher in Negroes of each sex, and alpha-2 globulins are only slightly higher in Negroes (entirely accounted for by the difference in females); Pollak finds this latter difference not statistically significant. Beta globulins are higher in Negroes, although this relationship is reversed to a small degree among males and the over-all difference is entirely accounted for by the high values in Negro females. Gamma globulins are markedly higher in Negroes of each sex, and this difference is somewhat greater in the series published by Pollak.

Just what the set of factors is which produces this lower albumin and higher globulin in Negroes is not clear. Differences in environment and exposure to disease must account for some of the effect, and certainly the environments of the two races in this study are different. However, even considering infectious diseases alone, it is well known that Negroes and Whites respond differently to identical innocula of specific organisms, and this may well be on the basis of biological differences inherent in each race. Certainly the evidence favors the conclusion of a genetic basis as being part of the reason the serum proteins differ between Whites and Negroes. In this study it must also be noted (see Table 9, p.46) that the age in Whites is 9.5 years greater than in Negroes. In view of the findings on age discussed below, the racial differences in the various globulin fractions might even be accentuated if this factor were removed. With the varied and large amount of further data available on the individuals in the New Haven Arthritis Survey, it would be a significant contribution to the world literature on the genetic basis for racial differences in serum protein to match a sample of Whites with those 56 Negroes having complete serum protein analyses and to examine this question more thoroughly.

Serum Proteins, Sex and Age. The general concensus among electrophoretic studies is that age shows a more significant effect upon serum proteins than does sex, and the decrease in albumin with increasing age is most pronounced while some globulin fractions variably show an increase with increasing age. Acheson and Jessop (1962) studied males over 65 and found significant increases with age in the gamma globulin fraction. Brackenridge and Caillag (1962) found a significant decrease

in albumin with age, with a rise in the alpha-1 and beta globulins (males higher) with a very significant increase with age among females throughout the age range, but a significant increase in males only between ages 18-49 years. Kirkeby also found significant regressions for total protein and albumin each decreasing with greater age. Nilsson and co-workers (1964) found with respect to sex differences that total protein and both beta globulin fractions are greater in males, with variable or no differences in the other protein fractions. They find the negative correlation between albumin and age the most striking finding and also strong positive correlations with age in the alpha-2 and beta-2 fractions. Samson et al (1965) found variable changes with age and sex in total protein, greater albumins in men up to age 50 with both sexes showing a steady decrease in albumin with age except for a transient rise in women aged 50-59, no age or sex differences in alpha-1 globulins, slightly greater alpha-2 globulins in men with variable and different age patterns in the two sexes, changing sex pattern in the beta globulin (greater in young women and older men) with gradual increases with age which are more pronounced among men, and gamma globulin levels which gradually increased with age (particularly in men), with some excess in men over women after the age of 49 years.

In the present study significant correlation coefficients indicate in Whites a greater total protein (1% level) and albumin (0.1% level) levels in males and greater alpha-2 (1% level) and, to a lesser extent (5% level), gamma globulins in females, with no significant correlations between sex and serum proteins in the smaller Negro group for any fraction. The correlation between sex and albumin remains significant at the 0.1-1.0% level in all age subgroups except over age 74 years. However, significant

correlation coefficients do not necessarily indicate that the mean differences are also statistically significant. Another significant correlation which was not further examined is the association in Whites of higher serum albumin with higher socioeconomic status in the combined sexes (1% level) and in males (5% level), but not in females.

In examining mean values for serum proteins, albumin is notably higher (both races) and total protein slightly higher (Whites only) in males, with total protein being higher in females among Negroes. Total protein levels change very little with age in either race or sex, and albumin changes little with age in Negroes, although in the Negro age subgroups the sexes are combined and relatively more females are present in the younger age group. Albumins in Whites, however, show very definite and progressive decreases with age, which increase in rate of change in each sex after ages 45-54, with the change in rate greater in males than in females.

Globulin fractions are each greater in females than males of the same race. In Whites this difference is least in the alpha-1 and beta globulins and relatively greater in the alpha-2 and gamma globulin fractions. In Negroes the sex difference is slight in alpha-1 globulins but relatively greater in the three remaining globulin fractions. The globulin changes with age in Negroes are a slight decrease in the alpha-2 fraction among older Negroes, a greater decrease in alpha-1 and beta fractions, and a slight increase in the gamma fraction. The interpretation of these changes in Negroes on the basis of age alone is open to question in that the proportion of each sex is different in the two age subgroups, more females being present among the younger. In view of the higher globulins in Negro females these decreases with age might be reversed

in the alpha and beta fractions, and the increase with age in the gamma fraction might in fact indicate a relatively greater change in Negro males than in Negro females.

In Whites the change with age is relatively little in the alpha-1 globulin. Alpha 2 globulin changes little until after ages 45-54 when in females it rises and then plateaus while in males it progressively increases to levels above those of females. Similarly, beta globulin increases in each sex after ages 45-54 with a drop in only the oldest female subgroup while at the same time male increases progress at a greater rate and surpass female mean levels. Except for a decrease in the youngest female age subgroup, gamma globulin changes little in either sex until after ages 45-54 when it begins to increase, but the change in males far exceeds that in females so that male levels are well above those in females by the oldest ages.

The findings of age and sex differences in serum protein levels described above and substantiated by the few available reports in the literature have to date no definitive explanation. Men and women have different genetic and endocrine heritages, lead different lives in terms of environment in the form of toxin exposure, stress, trauma, child-bearing and nutrition, have different metabolic patterns, and are subject to a relatively different spectrum of diseases, neoplastic, cardiovascular, infectious, and otherwise, which are not clearly related nor unrelated to the aforementioned factors. The progressive changes with age in both sexes of the serum albumin somehow reflect relative changes in intake and production, requirement, and destruction or loss of each protein throughout adult life.

The increases in each of the serum globulins after one passes the ages of 45-54 brings one to consider what distinct changes occur at that time. Prior to this age females usually menstruate and often bear children, both distinctly affecting their metabolic needs, and after which distinct hormonal changes take place. Jencks (1956) finds in pregnancy and the post-partum period a decrease in serum albumin and an increase in each of the four globulin fractions significant at the 0.1% level (1% level for gamma), with a greater degree of variation in each fraction compared with normal persons. Putnam (1960) reports a progressive drop in serum albumin during pregnancy (which does not return to normal levels until eight weeks post-partum) and a doubling of the beta globulin and a decrease in the gamma fraction. In males, in whom the rates of globulin change with age are even greater than in females, any hormonal change similar to menopause is neither nearly as abrupt nor as narrowly confined to specific ages. Chronic infection tends to produce the protein pattern of lowered albumin and raised levels of alpha-2 and gamma globulins, and cardiovascular disease (in patients with strokes, myocardial infarctions, angina, arteriosclerosis obliterans, rheumatic heart disease) may lower albumin and increase each globulin fraction (Jencks, 1956) so that these factors may account for a significant portion of the general change in means. Bronchial asthma may also affect each fraction this way (Jencks, 1956). However, examination of mean values need not reveal protein patterns in individuals. Furthermore, individual globulin fractions may be elevated (more rarely decreased) in a wide variety of disease states (Wall, 1958; Putnam, 1960; Jencks, 1956; and others). Alpha globulins may be increased in trauma, infection, fever, rheumatoid arthritis, cancer or pemphigus.

In nephrosis, in addition to the resultant hypoalbuminemia, increased alpha-2 and beta globulins may be present, including increases in both the lipid and protein components in these lipoprotein fractions. Beta globulins are also elevated in liver diseases (biliary cirrhosis, viral and toxic hepatitis, obstructive jaundice, cirrhosis), malignant hypertension, periarteritis nodosa, Cushing's syndrome, malaria, cancer, multiple sclerosis and sarcoidosis. Any condition stimulating the plasma cells of the reticulo-endothelium system may cause a rise in the serum level of gamma globulin. These cells are widespread in the body and are found in the bone marrow, liver, spleen, lung, thymus and many other organs (Andersen, 1964). Gamma globulins are certainly produced as specific antibodies to infecting pathogens, but are also produced in response to antigenic stimuli in the form of non-pathogenic microorganisms, foods and many other substances. Levels of gamma globulin are increased in infections, liver diseases, diffuse diseases of mesenchymal tissue, but also might be increased in old age partly as a reflection of the cumulative response to continued and varied antigenic stimulation by both exogenous and endogenous (autoimmune) antigens (Strehler, 1964).

The change in serum proteins described after ages 45-54 may reflect other physiologic alterations such as changes in the nutritional intake and differential absorptive powers of the gastrointestinal tract, changes in body weight and composition with decreased powers of synthesis or elimination and increased tissue catabolism. Strehler (1964) makes note that prominent globulin increases with age occur in those fractions possessing lipid prosthetic groups and having lower electrophoretic migration rates. It may also be due to a differential survival rate after the

age of 45 or to an increased prevalence of tissue destruction in continuing neoplasm.

In summary, in the present study serum proteins have differences according to age which are more important than those according to sex. Sex differences are more pronounced in serum albumin (males higher), with lesser differences in each of the globulin fractions (females higher), although these differences in globulins decrease or reverse in old age. The changes with age are more striking in the continuous decrease in serum albumin with an acceleration of this decrease after the age of 45 - 54, than is the change in alpha-2, beta and gamma globulins to higher levels after that same age. Therefore, sex and particularly age must be considered in examining serum albumin throughout adulthood, and age should be considered as a factor in the level of globulins after the age of 45 - 54. Some factors affecting serum proteins have been commented upon, but no conclusion can be drawn from the present analysis as to the reason for the serum protein changes. Further work on the basic data in this survey might be done by regression analysis of each serum protein fraction with age as an independent variable to determine its relation with serum protein variation. In addition, when remaining data from the survey are available, more specific information about those individuals responsible for the changes in mean values may be examined to see how they differ as a group from other individuals.

Uric Acid. Mean uric acid levels in the present study are higher in each sex than those found by enzymatic methods in the Tecumseh study (Mikkelsen et al., 1965). In the New Haven study, no apparent racial difference in serum uric acid is present. The sex difference (males

higher) is clear at all ages. In White males there is an increase of 1.23 mg/100 ml with age from the youngest to the oldest, but if the small samples (28 and 18) at these extremes are disregarded the change is measurably less. In White females, with the exception of small decreases at the two extremes of the age curve, uric acid increases smoothly and gradually as age increases. The correlation coefficients between age and uric acid in Whites are positive and significant for the combines sexes (1% level), for males (5% level), and for females (0.1% level). Among the six race and sex groups analyzed, social class and uric acid correlated significantly only in White females (1% level) with uric acids tending to be higher in lower socioeconomic classes. In Negroes, divided at age 35 years, there is an increase of 1.05 mg/100 ml in serum uric acid with age. However, if equal numbers of each sex were present in the two age groups, this difference would be expected to be decreased. Nevertheless, the correlation coefficients between age and uric acid in Negroes are positive and significant in females (1% level), males (5% level) as well as in the combined sexes (0.1% level).

Hemoglobin. Mean hemoglobin levels are higher in males compared with females of the same race, and higher in Whites when compared with Negroes of the same sex. The difference by sex in Whites is clear at all ages. In White males hemoglobin level is steady until ages 45 - 54, after which the mean falls progressively for a total change of 0.53 g/100 ml. The correlation coefficient between age and hemoglobin for all White males is negative and significant at the 5% level, but the change in means is small. The pattern in White females is different in falling to a low mean hemoglobin during peak child-bearing years (25 - 34), then

rising to a plateau starting at ages 45 - 54 and continuing through the remaining ages. In females the correlation between age and hemoglobin is positive and significant at the 1% level, but if the sexes are combined in Whites the correlation is not significant, apparently indicating that the opposing trends in the two sexes eliminate the statistical significance found individually. In Negroes the mean hemoglobin rises with age, but the older group contains relatively more males. The correlation coefficients between age and hemoglobin in Negroes are positive and significant at the 1% level in the combined sexes and in females, but no significance in males.

Interrelationships Among Serum Proteins, Uric Acid and Hemoglobin.

Correlation coefficients between total protein and each of its component parts (albumin and four globulin fractions) are consistently significant to a high degree. This is expected, although it is of some interest that the correlation coefficient between total protein and albumin, still highly significant, is numerically less than those between total protein and any globulin fraction. Furthermore, in most groups studied the correlation with total protein is greatest with gamma globulin and progressively less with beta, alpha-2, alpha-1 globulins and least with albumin. Among the globulins this is a similar order to that found in change of mean values with age, which by simple calculation does not in White males at least appear to be merely a proportional change in fractions of different concentration.

Other correlations found are that albumin has a significant negative correlation with each globulin fraction, with little variation, one fraction to the next. Also globulin fractions each correlate highly with one

another, and this correlation tends to be greater between fractions more adjacent to one another in electrophoretic separation, that is, alpha-1 correlates more highly with alpha-2 globulin than it does with beta or (least of all) with gamma fractions.

Similar findings in correlation between albumin and each globulin and also between any two globulins are reported in the Kristianstad Survey (Nilsson et al, 1964), as well as other correlations which may be relevant to possible causes for these phenomena. In that study, erythrocyte sedimentation rate (E.S.R.) was found to correlate negatively with serum albumin and positively with the various globulin fractions, and the globulin fractions were also found to correlate positively with the number of circulating leukocytes, mainly accounted for by the positive correlation between serum protein fractions and the number of polynuclear leukocytes. In view of the typical elevation in the E.S.R. and polynuclear leukocytes in response to infection or inflammation (whatever its degree of severity along the subclinical and clinical scale) sometimes together with the so-called "stress pattern" of change in the serum proteins characterized by a variable decrease in albumin and an increase of alpha-2 and often gamma globulins, and sometimes an increase in alpha-1 and decrease in beta globulins, some of the findings above may indeed be attributable to current infection. These correlations found among E.S.R., leukocytes and serum proteins may also give slightly more support to the hypothesis that the changes in mean serum proteins after the age of 45 - 54 (described in the previous section) may be in part due to chronic infection or tissue break-down.

Another set of findings in the Kristianstad Survey would indicate that increase in body fat might explain some of the change found after ages 45 - 54 in the present study. Nilsson found that body fat correlates positively at the 5% level with alpha-2, beta-1, beta-2 and gamma globulins in males and beta-2 globulins in females. Also in females body fat is negatively correlated with albumin at the 5% level and positively with gamma globulin at the 10% level. However, these are only clues as to what may in part be the basis for the changes and correlations found in serum proteins.

Examination of the correlation coefficients and multiple regression analyses in the present study reveal several significant relationships among the serum proteins, uric acid and hemoglobin. Significant positive correlations are present between not only the total protein and albumin as described above but also between total protein and uric acid, albumin and uric acid, hemoglobin and uric acid, hemoglobin and total protein, hemoglobin and albumin, but not between either uric acid or hemoglobin and any of the globulin fractions except quite sporadically. Another study (Holmes et al, 1951) has described a significant positive relationship between albumin and RBC count, without a significant relationship between total protein and RBC but a decrease in beta globulin as both the albumin and RBC count increased. In contrast to this last finding, Nilsson (1964) noted a significant positive correlation between either RBC or hemoglobin and beta-1 globulin, and a positive but nonsignificant correlation between either RBC or hemoglobin and the beta-2 fraction, but does not report the results of correlations between RBC or hemoglobin and total protein or albumin. Holmes (1951) tentatively offers that low dietary intake or protein may be responsible for both low albumins and low

albumins and low RBC count. Nilsson (1964) does not comment on the finding noted.

Acheson and O'Brien (1966) analysing the present data suggest that the smaller relationship between uric acid and hemoglobin in women than in men might be due to the female loss of red blood cells in menstruation. The Pearsonian correlation coefficients in the present study, however, while very highly significant in the combined sexes in age subgroups between 21 - 64, are significant in men only between ages 25 - 54 and in women aged 21 - 24 and 45 - 54. These latter findings may be a result of the smaller sample sizes in the age subgroups of individual sexes, but may also indicate that, in the absence of significant correlation between the two blood components in the female during the majority of menstruating years, causes other than menstrual blood loss are significant in the marked sex difference in mean levels of uric acid and hemoglobin.

In somewhat similar fashion, the correlation coefficient between uric acid and albumin is highly significant in the combined sexes for each age subgroup, except the very oldest, and is significant in females of all ages (5% level) or ages 25 - 34 (1% level), but in no male group studied. In the multiple regression analyses a significant dependence of uric acid on albumin is present in some sets of variables in males, but in females the dependence on albumin is even more significant than that on hemoglobin. Simple correlation between albumin and hemoglobin is significant at the 5% level in males only when all ages are combined, but between 5 to 0.1% levels in females excepting ages 21 - 24 and 45 - 54 and in the combined sexes in all ages 21 - 74.

There is also a significant correlation between total protein and uric acid which follows no age pattern in the present analysis, but which is more significant among females than among males, and again more significant when the sexes are combined. Total protein does not correlate significantly with hemoglobin in males, but between ages 25-44 or when all ages are combined correlates highly both in the combined sexes and in females.

The data therefore indicate that significant relationships exist between total protein, albumin, uric acid and hemoglobin, and that these relationships are different to a degree between the two sexes and between different ages. The fact that mean levels in albumin, uric acid and hemoglobin are each higher in males than in females may indicate that some of the relationships are a result of the mean differences by sex, but also may indicate that the differences by sex are in part a result of these relationships. However, in females the effect of albumin is greater than of hemoglobin on uric acid or of uric acid on hemoglobin, while in males uric acid and hemoglobin have greater dependence on one another than does albumin on either. The sex differences described, both in mean values and in correlations between blood components, together with different age patterns between the two sexes suggest that important events with respect to the factors studied are linked to a difference in females during the years of sexual fertility and menstruation from females after the menopause or males of any age. These might be the increased demands of pregnancy and child-bearing as well as menstrual blood losses on protein and hemoglobin production, the hormonal variations which occur with the menstrual cycles and with pregnancy as well as the more constant hormonal

change which takes place after the menopause, or even the differences in female nutrition and changes in weight and body fat content at various cycles or stages of life in contrast both within the female sex as well as between the two sexes. The number and frequency of pregnancies in a woman may be a significant factor in altering serum proteins, uric acid or hemoglobin.

The fact is that the relationships between these several blood components are present in each sex to a small but significant degree. It may yet be found that these blood factors or their metabolic breakdown products are directly linked to the synthesis of the other related factors, or that intermediate metabolites in the synthesis of one have a stimulating effect on the production of the others. Globulins and albumins, on the other hand, may compete with one another for available amino acids. The possibilities are many, and the question of what the nature of the relationship between factors is must await further investigation. Some of the questions can undoubtedly be approached through different analyses using the data in the present study as well as the additional data available from the New Haven Arthritis Survey. It is hoped that the findings of the present study will at least be a useful foundation for further research.

Conclusions.

1. Higher socioeconomic status in Whites is associated with higher serum albumin levels in the combined sexes and in males, and with lower serum uric acid levels in females.

2. A sex difference exists in serum protein levels which is most prominent in albumin (females less), but also present in each globulin fraction (females more); each of these differences is reversed in the oldest age group.

3. Aging is accompanied by a change in serum proteins which is greater than the differences found between sexes. Albumin progressively decreases with age, and alpha-2, beta and gamma globulins increase after age 45-54. Alpha-1 globulin changes little with age. In each case the change with age is greater in males than in females.

4. There is a difference in serum protein levels between Whites and Negroes. In Negroes albumins are lower and each globulin fraction is somewhat higher, most prominent in the gamma and beta fractions. Negro females show these globulin differences to a greater degree than do Negro males.

5. Uric acid is clearly higher in males than females at all ages. In males there is an irregular increase with age, while the increase in females is constant between ages 25-74. There is little racial difference.

6. Hemoglobin is clearly higher in males than in females at

1. Higher socioeconomic status in whites is associated with higher serum albumin levels in males and with lower serum uric acid levels in females.

2. A sex difference exists in serum protein levels which is most prominent in albumin (females less), but also present in each globulin fraction (females more); each of these differences is reversed in the oldest age group.

3. Aging is accompanied by a change in serum protein which is greater than the difference found between sexes. Albumin progressively decreases with age, and alpha-2, beta and gamma globulins increase after age 45-54. Alpha-1 globulin changes little with age. In each case the change with age is greater in males than in females.

4. There is a difference in serum protein levels between Negroes and whites. In Negroes albumins are lower and each globulin fraction is somewhat higher, most prominent in the gamma and beta fractions. Negro females show these globulin differences to a greater degree than do Negro males.

5. Uric acid is clearly higher in males than females at all ages. In males there is an irregular increase with age, until the increase in females is finished between ages 12-17. There is little racial difference.

all ages and in Whites higher than in Negroes. The sex difference in each race is approximately twice the difference found between the two races. Hemoglobin in males decreases slightly (both gradually and continually) with age, and in females is lowest between ages 25-44, after which it remains at a constant high level for that sex.

7. Total protein correlates positively and significantly with each of its component fractions, and this correlation is greatest with gamma and beta globulins.

8. Albumin correlates negatively and significantly with each globulin fraction, and these correlations are relatively equal with each globulin.

9. Uric acid or hemoglobin does not correlate significantly with any globulin fraction.

10. Significant correlations exist between uric and hemoglobin, albumin or total protein and between hemoglobin and uric acid, albumin or total protein. In males the correlation between uric acid and hemoglobin is greater than in females. In females the relationships are greater than in males between albumin and uric acid, albumin and hemoglobin, total protein and uric acid, and total protein and hemoglobin.

11. Multiple regression analyses in each sex using age, uric acid, hemoglobin, albumin and globulin fractions as variables reveal different patterns of relationship in each sex. With uric acid as dependent variable, small but significant

relationships are found (greatest to least) in males with hemoglobin, age, and albumin, and in females with age, albumin and hemoglobin. With hemoglobin as dependent variable small, but significant, relationships are found (greatest to least) in males with uric acid, age and albumin, and in females with age, albumin and uric acid. Globulin fractions do not contribute significantly to the variance in either uric acid or in hemoglobin.

12. No final conclusions are drawn as to the causes of the differences, trends and correlations described, although possible factors which may affect these findings are discussed.

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